Bio-Chemistry

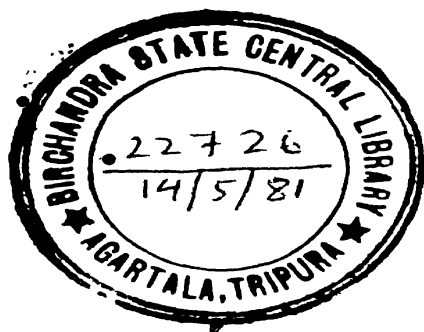## *Volume II*

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ONE

Cell and Organism

An individual organism may be considered as one unit of life whose function is to grow, to maintain itself and to reproduce. All organisms consist entirely of cells or cell products. This generalization is known as the 'cell theory' formulated by two German biologists Schleiden and Schwann in 1838. This theory became one of the corner-stones of modern biology.

Food is taken and used either to build up body tissues or to provide energy for various body processes. The food may therefore provide building blocks or fuel for the body. An individual animal is not, however, a homogeneous mass of tissue. The work load is distributed and the organs become specialized to perform particular jobs assigned to them. The skeleton supports the body, the muscles move it around, the heart pumps blood, the kidneys excrete the waste products, the lungs provide oxygen and the nerves and hormones control the activity of other organs. Even the specialized organs are not composed of a uniform collection of cells. Most organs contain cells of several different types. The pancreas is composed of a tissue secreting digestive enzymes and its islets of Langerhans secrete hormones. The islets themselves are not homogeneous, its α-cells secrete glucagon and β-cells, insulin.

The English biologist Robert Brown discovered nuclei in cells in 1831. The Bohemian biologist Purkinje assigned the name protoplasm in 1839 to the living substance out of which cells are made. Virchow propounded in 1855 that new cells can arise only by the reproduction of already existing o~ . This was an important recognition of the continuity of life, of wth and development of organisms and of evolution.

he cell diameters vary considerably from about $0.2\,\mu$ to several

millimeters ($\mu = 1$ micron $= 1/1000$ cm). The size of the vast majority of cells is remarkably uniform ranging generally between 0.5 to 15 μ in diameter. A size too small would not have provided enough space to accommodate its necessary parts and a size too large would have increased its maintenance problem and reduced the operating efficiency. With the increase in cell size, its surface enlarges as the square of its radius and the available surface area determines the uptake of nutrients and elimination of waste products. The cell volume, however, increases with the cube of the radius and the volume determines how much mass a cell must keep alive. As the cell continues to enlarge, its mass will eventually outrun the food-procuring capacity of its surface and the cell growth ceases. The actual size of a cell appears to be dependent in part, on the surface-to-volume relations.

NUCLEUS AND CYTOPLASM

Most enzymes function inside cells rather than outside them, notable exceptions being the digestive juices. The organism as a whole is divided into specialized organs and a cell into organelles each having a special function to perform. The fundamental subdivisions of most cells are nucleus and the substance that surrounds the nucleus, called cytoplasm. The nucleus is bounded by a nuclear membrane and the cytoplasm by a cell membrane, which is also known as plasma membrane. A cell wall surrounds the cell membrane in many cases. Most cells contain a single nucleus each but there are exceptions also. Membrane-bounded nuclei lack in bacteria and blue-green algae as also mammalian red blood corpuscles and several types of plant cells lose their nuclei with maturation.

The complexity of cellular structure has been revealed by electron microscopic study. The cell is surrounded by a cytoplasmic membrane and in most cells, the cytoplasm contains a series of membranes—the endoplasmic reticulum which is thought to bound channels through the cytoplasm. In the centre is the nucleus containing a mesh work of densely staining deoxyribonucleoprotein, the chromatin. The chromatin during preparation for cell division condenses to form the chromosomes which contain all the DNA of the cell. A double membrane surrounds the nucleus and is pierced at intervals by pores and contains one or more dense, spherical bodies, called nucleoli (little nuclei) rich in RNA. The complexity of the endoplasmic reticulum varies considerably with the cell type. In some cells it is covered by small granules which are electron dense and are known as ribosomes, the sites considered for protein synthesis.

MITOCHONDRIA

Also within the cytoplasm are granules called mitochondria which may

appear as rods, spheres or filaments, surrounded by a double membrane. The dimensions of mitochondria range between 0.5 to 5 μ by 0.3 to 0.7 μ. Each membrane probably consists of two outer layers of protein molecules separated by an inner double layer of lipid molecules. The inner membrane is folded into complex shapes known as cristae and carries enzymes on its surface. Cells which use up large quantities of oxygen have many mitochondria with numerous and complex cristae. The mitochondria (there are about 400 in a liver cell) contain the enzymes responsible for oxidative phosphorylation and are the site of production of high energy compounds, such as adenosine triphosphate (ATP) in the cell and are designated as the power plants of the cell.

CHROMOSOMES

Chromosomes are conspicuous only during cell reproduction when they become thickly coated with additional nucleoprotein. Such coats are absent at other times when the chromosomes are very fine filaments. The exact number of chromosomes in each cell nucleus is an important species-specific trait. Cells of human beings contain 46 chromosomes each. The cells of every other type of organism have their own characteristic chromosome number. Functionally the chromosomes are the carriers of the genes, which are the ultimate controllers of cellular processes.

RIBOSOMES

The ribosomes consist of about equal amounts of protein and RNA and, together with transfer RNA (tRNA), messenger RNA (mRNA), activated amino acids, enzymes and other factors, they participate in the complex process of protein synthesis. Ribosomes also occur free in the cytoplasm, unattached to reticular membranes. All the DNA is in the nucleus while RNA is found in the nucleus, particularly the nucleolus, and the cytoplasm. In the process of protein synthesis ribosome is attached to c end of a molecule of messenger RNA (mRNA) and starts moving along the mRNA and with the transfer RNA (tRNA) molecules and certain other factors, translates the nucleotide sequence of the mRNA into the amino acids sequence of a code-specific protein.

P otein synthesis differs from the manufacture of other cellular components. A cell is not able to use just any newly made proteins, only specific proteins can be used by it to maintain its own special characteristics. This problem does not arise with other kinds of compounds. A cellulose molecule, for example, consists of identical glucose units and is structured like any other cellulose molecule containing the same number of glucose units. A protein molecule, on the other hand, is composed of different kinds of amino acid units and a random linking of these units would give rise to a polypeptide chain quite different from each

other. A specificity control is therefore necessary for protein synthesis. Such specificity control is exercised by the genes of a cell, the DNA of chromosomes. The different genes in a cell carry different messages and proteins are manufactured by a cell as the genetic instructions dictate. The chromosomes in the nucleus contain the genes but the sites for protein synthesis are the ribosomes in the cytoplasm. Genetic instructions from the chromosomes are transmitted to the ribosomes by RNA. RNA is produced by the chromosomes and in the process the chemical message of DNA becomes incorporated, or transcribed, in the structure of RNA. The new RNA molecules leave the chromosomes and diffuse to the cytoplasm, where they eventually reach the ribosomes. Amino acids are joined together here as proteins in accordance with the genetic instruction provided by the RNA molecules (see Fig. 1.1).
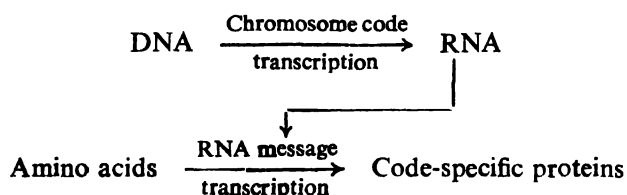
$$DNA \xrightarrow[\text{transcription}]{\text{Chromosome code}} RNA$$

$$\text{Amino acids} \xrightarrow[\text{transcription}]{\text{RNA message}} \text{Code-specific proteins}$$

Fig. 1.1

The endoplasmic reticulum and mitochondria are embedded in the general cell matrix or cell sap containing the low molecular weight transfer RNA (tRNA).

LYSOSOMES

The cell cytoplasm also contains in most cells, small organelles, known as lysosomes. It was first described by De Duve in 1955 and has attracted a good deal of interest. These organelles are tiny membrane-bounded sacs or vesicles. They contain digestive enzymes that are released into the cytoplasm when the vesicles burst open. The enzymes found inside the lysosomes are primarily destructive in nature and can break down most of the complex molecules present inside cells. They are normally kept in check by the membrane that surrounds the organelle. The presence of these enzymes suggests that they are released to destroy unwanted or dying tissue. Lysosomes appear to be involved in the regression of the tadpole's tail, in the involution of the mammary gland after lactation and in the destruction of the uterine wall during menstruation. But evidence for these roles is not conclusive.

It may be that the lysosomes form the cell's own digestive system involved in the breakdown of unwanted proteins and other compounds into their constituents before they are rebuilt into new types of complex molecule. The lysosome enzymes seem to be involved in the various normal decomposition processes in a cell—chemical breakdown of nutrients

prior to their utilization, breakdown of cellular organelles prior to their reconstruction or remodelling, breakdown of foreign particles and so on. Lysosomes thus appear to play a role in tissue maintenance—old cells are replaced by the new formed through cell reproduction. The enzymes in lysosomes include cathepsin, which break down proteins, ribonuclease, deoxyribonuclease, β-glucuronidase and acid phosphatase. The dissolution of damaged or dead cells during autolysis is attributed to lysosome enzymes.

PLASTIDS

Several other types of granule may be present in the cells. These may be pigment granules such as plastids, occurring in the cells of most algae and all green plants. On the basis of their pigment content, plastids can be classified into three kinds: the first, in which there is no pigment such as leucoplasts serving as starch-storing organelles as in the cells of potatoes; the second chromoplast contains a variety of carotenoid pigments such as carrots and tomatoes their carotenoid colours being localized in chromoplasts; and the third chloroplasts although containing carotenoid pigments owe their colours due to the presence of a large quantity of green pigment chlorophyll which gives the characteristic green colour to leaves and other plant parts. Chloroplasts (4 to 6 μ in diameter) have a protein framework arranged in parallel layers with smaller organelles, called grana, in between. All of these contain protein layers and the space in between contains DNA, enzymes, chlorophyll and others—the whole machinery for food manufacture. Grana in fact are the structural and functional units for photosynthesis.

GOLGI BODIES

The Golgi bodies are exceedingly complex organelles made up of an interlacing network of fibrils and vesicles. These bodies are seen as stacks of thin, plate-like layers under electron microscope. The precise function of Golgi bodies is uncertain but it may be associated with the final stages of the formation of secretory granules. They are particularly conspicuous in actively secreting cells.

CENTRIOLES AND KINETOSOMES

A small granule called centriole is located near and in some cases inside the nucleus. Some algae, some fungi and all animal cells contain centrioles. Most of the cells of plants do not contain centrioles. These granules are specifically involved in cell reproduction. Another type of granule is basal granule or kinetosome, which occurs in cells having surface flagella or cilia. Kinetosomes are concerned with such functions as to anchor and control the motion of these surface structures. Both of them have a common, complex structure.

ADDITIONAL STRUCTURES

Cytoplasms generally contain additional granules and fluid-filled droplets called vacuoles. These organelles function in a large variety of processes, in the transport of nutrients from cell surface to the interior (food vacuoles) or of finished products in the opposite direction (secretion granules); as places of storage (starch granules, glycogen granules, fat vacuoles, water vacuoles, pigment granules) or for carrying waste materials to points of elimination (excretory vacuoles).

A variety of long, thin protein fibrils may also occur in the cytoplasm. Some examples are contractile myofibrils or conducting neurofibrils. Peroxisomes are somewhat smaller than mitochondria. They correspond to the micro-bodies of liver as revealed in electron micrographs. The enzymes, urate oxidase, D-amino acid oxidase and catalase are present in these micro-bodies. Hydrogen peroxide is formed by the first two enzymes whereas the third is concerned in its destruction.

A typical animal cell is shown in Fig. 1.2.

The cells of the animal body vary widely in size and form depending on their adaptation to perform a variety of specific functions. The tissue cells which have acquired a fixed location in the body, assume polyhedral, columnar, flat or spindle shape. The nerve cells with its processes sometimes several feet long, is probably the most aberrant type. The laws that control or limit the size of the cell and the body size are not well understood. Large cells are found in some groups of animals. This however does not mean that large animals have large cells and small animals, small cells. The size of the individual in general is determined by the number of its cells and not by their size.

CELL LIFE AND CELL DEATH

The living matter is believed to be constructed in accordance with the same basic principles as is the physical world in which it exists. In keeping with the laws of thermodynamics, living systems might be expected to decrease in complexity gradually as energy is always involved in the maintenance of high degrees of organization and yet the trend is toward more complexity in the evolution of living systems.

The answer to this is the continual input of free energy from the sun into living systems. This energy was available in the synthesis of the simplest of organic substances and now continuously used to maintain and extend the organization of living things. The energy of the sun is not used directly by many higher organisms which however feed on lower forms that do. Cell enzymes catalyze the chemical reactions within the cell efficiently to ensure the process of cell growth with remarkable rapidity. The process of the construction of macromolecules, which is the process of growth, is called anabolism. For the breakdown of its components, the cells also use mechanisms called catabolism. The energy stored by the
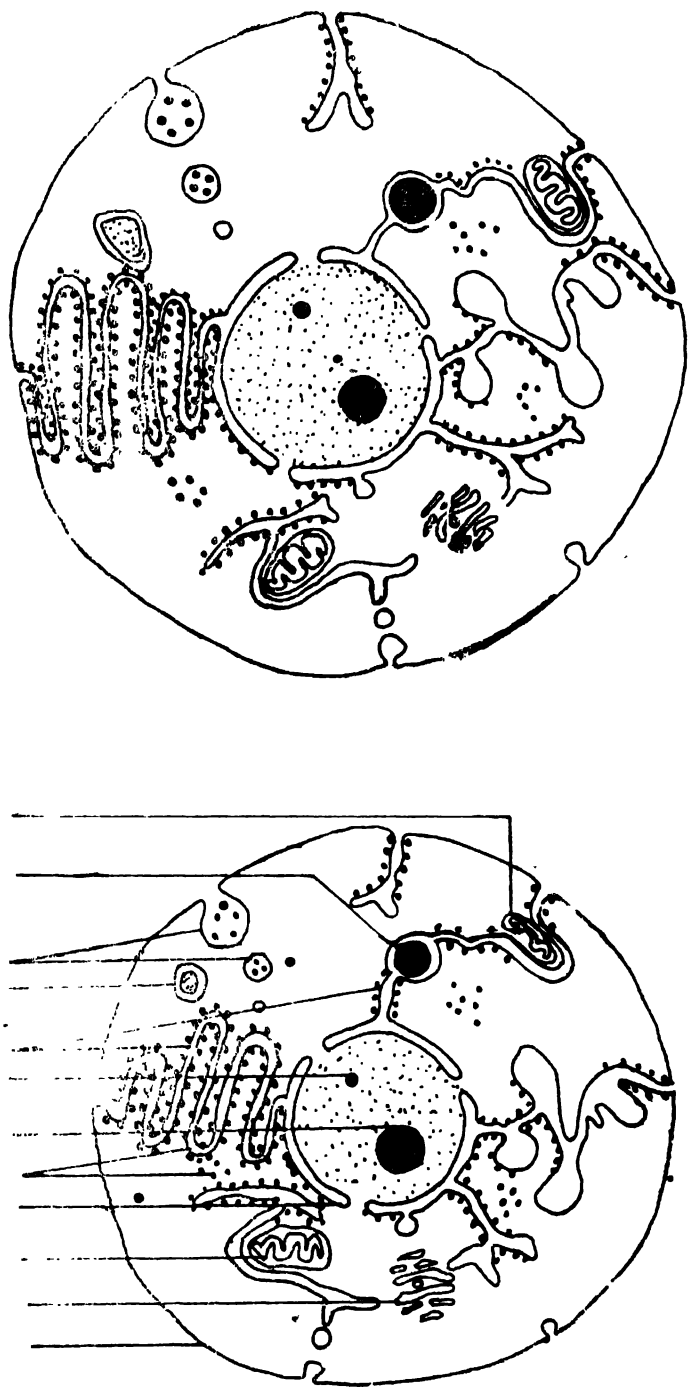
Fig 1.2   Schematic diagram of a typical animal cell
(From J. N. Davidson) •

cells is released by these catabolic mechanisms, such as the breakdown of fatty acids, and are a necessary part of the ongoing activities of the cell. Both anabolism and catabolism are important for maintaining the proper functional levels of materials within the cell. Failure of the proper enzymatic degradation of cellular constituents gives rise to several important diseases, called storage diseases and not as a result of lack of anabolic activity within the cell. Specific cell components under these conditions, accummulate within the cell in abnormal amounts seriously interfering with cell function.

Cell death is an integral part of the growth of tissues and organs. Large number of cells may die during the development of some tissues. An initial overproduction of certain cell types takes place in these processes but only those required for the functional needs of the tissues will survive. Local cell death (necrosis) occurs normally in the body or may be result of influences which cause disease, such as trauma and inflammation. The necrotic tissue is recognized morphologically by the altered structure of the cells and intracellular substance. Proteins coagulate in the cytoplasm which appears flocculated or in the nature of a fibrous network. Obliteration of the cellular boundaries makes the cell appear fused. Or the cell may liquefy, swell and finally burst, the process being known as cytolysis, the liquefaction is often preceded by the appearance of numerous fat granules. These proteolytic processes are in many cases due to intracellular enzymes liberated after the death of the cell, a process called autolysis. The nucleus, in a similar way, shows various forms of structural disintegration. The chromatin may contract into a dense, deeply staining irregular mass, a process called pyknosis. It may break up into a number of small pieces with obliteration of nuclear boundary, the process being known as karyorrhexis. Or it may gradually disappear as revealed by the loss of its staining capacity, a process called karyolysis.

Viruses

Infective agents which multiply inside the cells of susceptible animals and are rerponsible for such diseases as small-pox, poliomyelitis, influenza, etc., are called viruses. They are very small in size ranging from 20 to 200 nm. Most of them consist of only one molecule of RNA or DNA surrounded by a protein coat. Virus multiplication takes place as a result of the genetic information carried by the nucleic acid. The nucleic acid is protected by the protein coat which also confers a number of specificity on the virus particle.

All viruses depend entirely on the host cell for energy (ATP) and a supply of precursors such as amino acids and nucleotides, necessary for the synthesis of new virus particles. Virus particles are quite inert outside the cell.

Most living things, animals, plants and microorganisms, are susceptible

to viruses. Viruses which infect bacteria are known as bacteriophages. The replication of viruses involve five stages—absorption, penetration and uncoating, synthesis of virus components, assembly, and release. As E.B. Wilson put it,

> Life is an unbroken series of cell divisions that extend backward from our own day throughout the entire past history of life.... It is a continuum, a never-ending stream of pɾ ɔtoplasm in the form of cells, maintained by assimilation, growth and division. The individual is but a passing eddy in the flow which vanishes and leaves no trace, while the general stream of life goes forward.

Cells arise from the division of pre-existing cells. There are an estimated 10^{14} cells that comprise the adult human body and all of them are derived from just one cell, the fertilized egg. The proteins in the cell determine its structure and specificity and mRNA directs the assembly of these proteins. The genetic material, the DNA, is contained within the nucleus and its code is carried by the mRNA. The newly formed cells must therefore have exact replicas of the parent DNA complement. The parent cell must duplicate exactly its DNA and then divide precisely and distribute it to two daughter cells. The process of duplication of DNA molecules is called replication and the mechanism by which the replicated DNA is divided in order to supply each daughter cell with its complete and exact complement of hereditary material is known as mitosis. Karyokinesis indicates the division of nuclear material and cytokinesis denotes the division of cytoplasm.

MACROMOLECULES IN CELL STRUCTURE AND FUNCTION

The molecular components of living systems can be classified by their functions. Metabolism of the cell involves most of the various molecular species, these molecules have intermediate roles in the production, utilization, and storage of energy. The other compounds confer structure and form, store the cell's heriditary information, catalyze metabolic reactions, organize the components, and carry out any other cellular functions. The first class of molecules consists almost entirely of small compounds and the second involved with every thing other than the actual metabolism of the cell, are uniformly of large size. The bulk of the molecules in a living cell are large. The concept of single molecules of relatively large size was introduced in the 1920s by Staudinger who gave the name macromolecules to describe them. Macromolecules of definite structure result from the conversion of well-defined small molecules by a process of controlled condensation reactions. The macromolecules are composed of repeating units linked by covalent bonds. The single repeating unit is commonly referred to as the monomer unit and the macromule composed of many monomers strung together is known as

polymer and the process by which monomers are converted to polymers is called polymerization.

Most of the biologically significant macromolecules are polymers. Many types of macromolecules have the striking feature in their malleability. Although such micromolecules often can exist as solids, they can also be distorted or are elastic like that of a soft rubber. The living cells have utilized the property of flexible solidity to great advantage. Through the use of macromolecules, cells have produced a cell envelope which not only gives the cell its form but also prevents escape of important substances and provides protection from the environment. This capsule, although like a solid, is not brittle or rigid. On the contrary, it is readily deformed and may be moved about without apparent damage. Muscle tissue is composed primarily of cells having a set of macromolecules capable of forming long fibres.

Biologically significant macromolecules have another important property—its ability to confer structure to a system while essentially remaining dissolved in the solvent, which produce very viscous solutions or even actual gels in some cases. This allows the establishment of a network through which diffusion of the solvent takes place while the macromolecules remain unperturbed. Living cells contain many structures which remain more or less fixed and yet substantially dissolved in the surrounding water environment. The small water soluble molecules are allowed to diffuse freely between structures and interact with the appropriate macromolecules. The most obvious common characteristic of macromolecules is their relatively large size. All cells are surrounded by a membrane containing pores of restricted size, which prevents the macromolecules being lost to the environment.

Biopolymers

Macromolecules from living systems, the biopolymers, can be divided into three distinct chemical groups—polysaccharides, nucleic acids, and proteins. Polysaccharides and nucleic acids in most cells have only a few specialized functions; proteins, however, are involved in an extraordinary diverse set
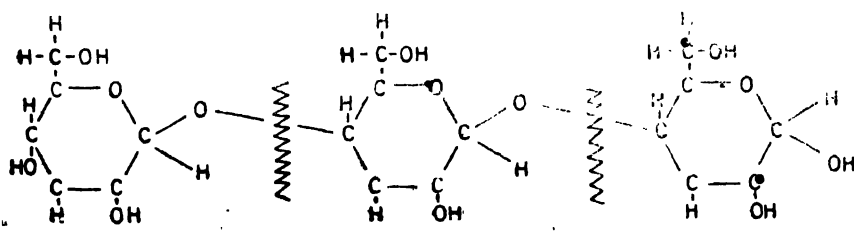
Structure of cellulose

of roles. All these macromolecules are mixed or irregular polymers, consisting of various monomer units linked together in a regular repeating manner. Polysaccharides are formed from a number of sugar moieties bounded together. The important polysaccharide cellulose is the prime component of plant cell walls. Very little is known about the structure of polysaccharides, other than their molecular composition   They might have a three-dimensional configuration which is under intensive investigation.

Nucleic acids are irregular biopolymers and are composed of monomer units called nucleotides. Nucleotides are composed of phosphate attached to a five carbon sugar (pentose) moiety which in turn is linked with a cyclic compound— either a purine or a pyrimidine base. When the sugar is deoxyribose, the biopolymer is called deoxyribonucleic acid or DNA and with ribose sugar it is ribonucleic acid or RNA. DNA and RNA constitute the two kinds of nucleic acids composed entirely of their respective sugars.

Structure of nucleotides
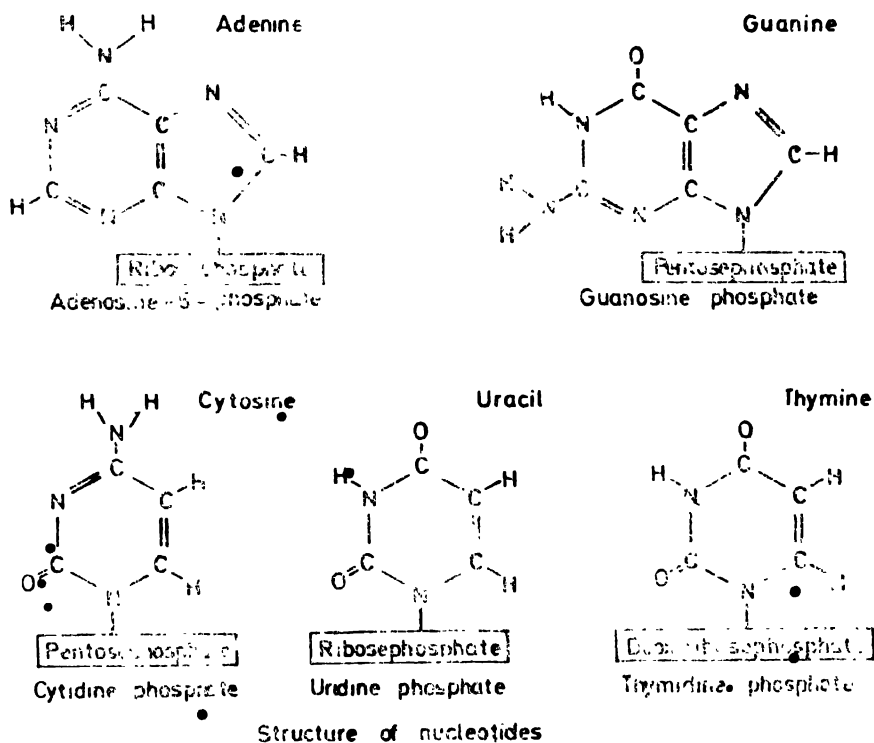
There is no species of nucleic acid which contains both types of sugar moieties. The linkage between monomer nucleotides is through a phosphate group called phosphodiester bond and always occurs between the 3' position of one sugar and the 5' position of the next. There are no cross linkages between nucleotide chains that are of covalent character and that have proved biological function. There are, however, very important noncovalent (weak) interactions, which take place between and

within chains. These interactions are highly specific, and they lie at the very root of the function of the nucleic acids. Hydrogen bonding appears to occur between certain purines and pyrimidines. The purine adenine is bonded to the pyrimidine (this is the case in DNA; in RNA uracil takes the place of thymine) and that guanine forms hydrogen bridges with cytosine. These interactions cannot occur with any other arrangement of bases.

The specific hydrogen-bond interactions between base pairs appears to be extremely important in the postulation of the three-dimensional structure of the nucleic acid molecules. The three-dimensional structure of DNA is that of a double helix in which two individual DNA chains are woven around one another (see Fig. 1.2). The two chains are bonded together by the specific base pairs. This structure of DNA has important functional implications.
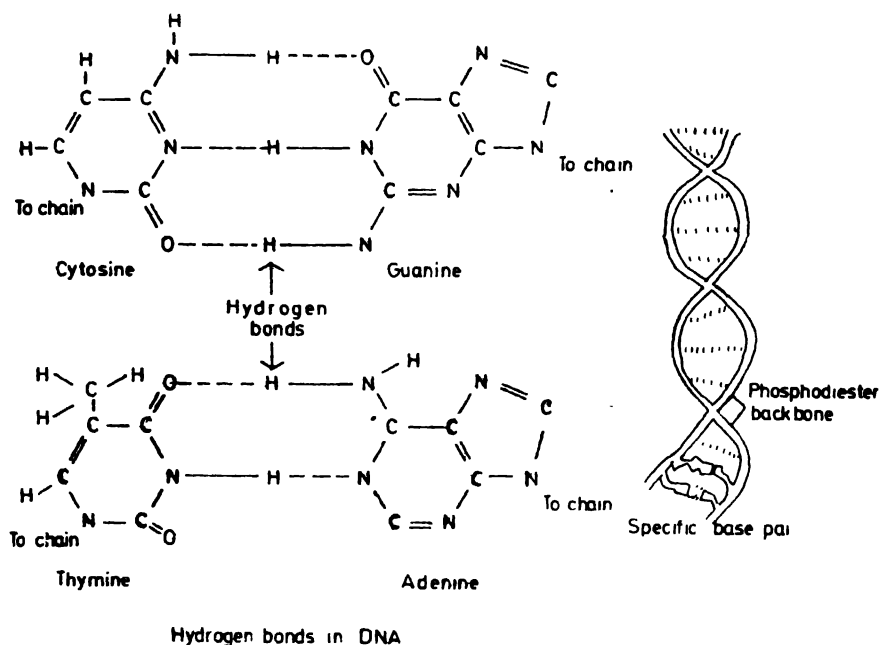

Hydrogen bonds in DNA

Fig. 1.2   The double helix of DNA

The three-dimensional structure of RNA is less well defined. Proteins are also irregular polymers. Most proteins consist of linear chains made up of 20 different monomer units called α-amino acids, which are bonded together through peptide linkage. Each protein is characterized by a unique sequence of amino acids. Various types of covalent cross linkage may be present in the protein. Protein molecules are responsible for the most important processes of a living cell. The different species of cells are identified by their protein content. A bacterial cell is fundamentally different from an animal cell by virtue of its structures and functions. All this is due to difference in their protein contents which serve to distinguish

one from the other. Bacterial cells divide to produce more bacterial cells, which is the case with all other cell types. The problem of heredity is, therefore, basically as to how the information inherent in a given cell is translated into its distinctive protein complement.

A specific sequence of nucleotides along the DNA molecule is transcribed into an RNA molecule with an exactly complementary sequence. The RNA molecule is called the messenger RNA (mRNA), which appears to exist in a single-stranded form. The messenger RNA molecule then gets decoded and translated into a protein molecule. The amino acid sequence of the protein molecule is specified by the nucleotide sequence of the messenger RNA. A hydrogen bond specified interaction takes place between the RNA molecule and an individual transfer RNA molecule (tRNA) carrying a particular amino acid at the ribosome surface or site. The nucleotides in the DNA ultimately specify a single amino acid in the protein. The protein chain is formed when two amino acids link together one at a time, at two such sites on the surface of the ribosome. The ribosome then moves on down the mRNA progressively opening sites for the next tRNA to attach and allowing the next amino acid to link into the growing chain. The individual steps involve starting the chain, completing the chain, moving the ribosome, forming the peptide bond and so on and are extremely complex.

The ribosome is composed of three different molecules of RNA, named ribosomal RNA and about fifty different protein molecules. It is likely that the RNA molecules help organize the fifty or so protein molecules to give them their unique quaternary configuration. The proteins themselves are more intimately involved in many of the complicated functions of the ribosome, which is relatively a very large structural unit composed of both RNA and protein and plays a central role in the process of information translation or protein synthesis.

Human Genetics

The hereditary material, DNA, is carried by the chromosomes in man as in other organisms. Somatic (body) cells, which have two of each type of chromosome, are diploid. In mitosis each chromosome is replicated and is represented in each of the two daughter cells. Gametes, or germ cells, which have one of each type of chromosome, are haploid and are produced in the process of meiosis. Segregation, assortment, and recombination occurring in the meiotic process are the basis of the characteristics of inheritance.

Apart from the gametes, every human cell contains 23 pairs of chromosomes, one member of each pair having been derived from the mother and one from the father. The two chromosomes in 22 of these pairs appear to be identical. These 22 pairs are the autosomes and the two members of each pair are said to be homologous. The 23rd pair

constitutes the sex chromosomes. In females the two are identical and both are called X. The male possesses one X chromosome and the other much smaller Y chromosome. .Complete chromosome pictures, known as karyotypes, of a normal male and a normal female are shown in Fig. 1.2.

DNA is the main component of chromosomes. Each chromosome carries a large number of DNA units each of which carries out a specific task during the development and operation of the cell. These DNA units are known as genes. Genetics has been defined as the science of differences or the science of variation. A single cell, the fertilized egg, gives rise to the many cell types of the mature organism. The fertilized egg divides rapidly, forming first a ball of cells called a morula. The mass of cells later develops a cavity and is termed a blastula. In mammals this becomes embedded in the uterine wall and is nourished by the maternal tissues. Three classes of cell exist in the embryonic germ disc of the blastula. They are: the ectoderm or outside layer, the endoderm or inside lining, and the mesoderm, the cells between these surface layers. The organization and development of these three layers of embryonic tissue are of fundamental importance. About a hundred kinds of cells will develop from these layers and their interaction to form the adult mammal. The process of functional and structural specialization of these cells is known as differentiation. In the process of differentiation, cells make a series of small shifts—cell of the blastocyst becomes a cell of the endoderm which proliferates to make more cells of its kind, these cells then become part of either the gut wall or the lung, this is followed by a third shift, the cell becoming either absorptive or secretory. In this manner the fate of the cell is set and the cell is said to be determined and becomes structurally differentiated to perform its specialized functions. The genetic material in the cell nucleus, the gene or cistran, determines this orderly development. The basic codes in the genetic material do not change with development. As the cells are progressively determined the genes are said to be differentially expressed. The different regions of the genome are turned on and others are turned off as cells develop. ,

Each structural gene carries information about the amino acid sequence required to make a particular protein or enzyme. A control gene does not carry a plan of protein structure but is capable of controlling the operation of structural genes or of other control genes. The alterations in the use of genetic material during development are not entirely preprogrammed within the cell but are influenced by interaction with other cells. As soon as the organism becomes multicellular, the cells begin to react with one another. The control genes may be able to control not only their own chromosome but the homologous chromosomes as well.

PHENOMENA OF HEREDITY

Organisms produce offsprings according to instructions provided by genes

and through the inheritance of genes by successive generations construction of new life becomes a nonrandom, controlled process. The survival potential of an organism is determined by what it inherits in large measure, heredity therefore has adaptive significance. But organisms do not inherit strong muscles, green leaves, red blood, or any other trait. They inherit the genes and all the other contents of reproductive cells. Under the control of the inherited genes, visible traits develop. The result of such heredity in an adult is reflected in the li'eness to its parents in certain major respects and variation from parents in many minor respects. When the variations are not lethal or do not cause infertility, the organism will survive and pass on its genes to following generations.

The unit of genetic information is gene or cistron. It is a segment of the DNA molecule containing on the average about 600 base pairs and the genetic message is carried in the sequence of bases along the DNA strand in double helix coiled tightly in each chromosome thread, written in the four letter language of the four bases. The message is transferred to messenger RNA in the process of transcription. The mRNA carries it to the ribosomes on which it is translated into the 20 letter language of the amino acids in the proteins. The genetic information is carried by the particular section of the DNA containing the gene or cistron for the correct sequence of amino acids in the polypeptide chain in one particular protein (or enzyme). This embodies the principle—one gene and one polypeptide. Each gene has its counterpart in the corresponding locus on the homologous chromosome for any particular characteristic. These two genes form an allelic pair. When the pair carry genes with the same characteristic which may be tallness, the individual is designated as homozygous with respect to that character. In case one of the pair carries tallness and the other shortness the individual is heterozygous.

Modern studies of heredity began in the last half of the nineteenth century by the Austrian monk Gregor Mendel (1882–84). He discovered two basic rules that laid the foundation for all later advances in genetics. The rules or laws as discovered by Mendel can be stated in modern terminology as: (a) law of segregation (b) law of independent assortment. Mendel crossed tall (*TT*) pea-plants with dwarf (*tt*) ones. All tall plants resulted in the first generation. They all possessed the *Tt* pair of allelic genes, *T* dominating over *t* and *t* being recessive. From these *Tt* plants by self-fertilizing, he obtained one quarter dwarfs and three quarters tall plants.

According to the law of segregation, the genes do not blend but behave as independent units. They pass intact from one generation to the next. They may or may not produce visible traits depending on their dominant characteristic. The genes segregate at random producing predictable ratios of visible traits in the offspring. The law of independent assortment states that the inheritance of a gene pair located on a given chromosome pair is not affected by the simultaneous inheritance of other gene pairs located on other chromosome pairs. That is, two or more traits produced by genes located on two or more different chromosome pairs assort,

independently, each trait is expressed independently as if no other traits were present.

Genes on the same chromosome may stay together as a gene string or they may be separated by crossing-over-interchanging of corresponding segments of homologous chromosomes. This reassortment of genetic material results in the difference in traits in the members of a succeeding generation and among themselves. Some of the offsprings may have a combination of characteristics with an enhanced survival value while others with a reduced value. The latter are eliminated by the process of natural selection. In this way a species adopts to changes in its external environment.

A large number of genes present in the chromosomes of each individual plant or animal, become assorted during development of the germ cells and the zygotes produced by their union must vary enormously in constitution. Any two children in a family are not absolutely alike unless they arise from one fertilized ovum. Such individuals are designated as monozygotic or identical twins.

THE OPERON

Jacob and Monod suggested a mechanism in 1961 based on the generally accepted assumption that the genetic information of the organism, encoded in the nucleotide sequence of DNA is transcribed into mRNA. The nucleotide sequences of mRNA are translated into the amino acid sequence of a specific polypeptide in union with ribosomes. According to Jacob and Monod, the synthesis of mRNA on the gene is regulated by specific repressors which are products of other genes, called the regulator
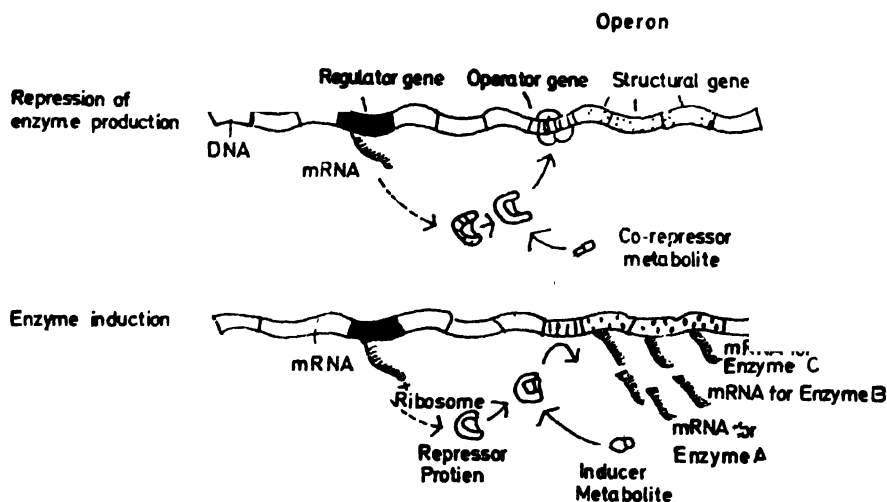

Fig. 1.3   Gene control (from Bailey's *Textbook of Histology 1971*)

genes. The repressors probably act by becoming engaged with the operator site of a group of genes. The operator together with the structural genes it controls, is known as the operon. When the operator site is open, all of the genes of the operon synthesize mRNA, and when it is closed by the repressor, none do. The concentration of small molecular weight metabolites within the cell influences the affinity of the repressor for the operator site. [F. Jacob and J. Monod, 1961, 'Genetic Regulatory Mechanisms in the Synthesis of Protein', *J Mol. Biol.*, Vol. 3, pp. 318–56.] The manner in which enzyme could be induced in a cell at the level of the gene can be explained with the help of this concept.

Operon Concept

A regulator gene occupying one site on DNA strand controls the production of a repressor protein. This protein in combination with a corepressor metabolite, inhibits the activity of an operator gene on another site of the DNA strand. The repressor protein is unable to block the operator site in presence of an inducer metabolite. This operator gene controls the activity of adjacent structural genes. mRNA molecules involved in enzyme manufacture assemble on adjacent structural genes.

GENES IN THE INDIVIDUAL

The genetic constitution of the individual is called genotype. The phenotype is the character or trait or the composite of traits or characters that is capable of being observed. The distinction between the two is that between character and reputation. Genotype and character are what one really is and phenotype and reputation are what one appears to be. Some hereditary disorders display dominant inheritance in some families, autosomal recessive inheritance in others and a X-linked inheritance in yet another. Elliptocytosis (oval-shaped erythrocytes), an autosomal dominant trait, is determined in some families by a gene rather closely linked to the Rh blood-group locus.

Genetic heterogeneity is the term applied to the situation in which more than one genetic cause leads to the same or very similar phenotypes. Sometimes the heterogeneity is allelic and sometimes nonallelic, the distinct genes resulting in a similar phenotype may be at the same locus or at different loci.

A trait may be highly variable from one person to another. Genetic traits that are subject to considerable modifications by the effects of genes other than the one primarily responsible for the trait and also by environmental influences may not be recognizable in some individuals in spite of the fact that they have the gene or gene pair causing the trait. In such cases the trait (or the gene) is said to be nonpenetrant.

Cells are adapted to the specific functions which they are required to perform in the different tissues and they are therefore often highly differentiated morphologically. This process of differentiation begins

during the embryonic period and continues during growth until the adult state is reached. There is, however, progressive loss of ability to multiply with increasing specialization. In some tissues, such as epidermis and bone marrow, certain cells remain undifferentiated and these can multiply rapidly. All the cells in glandular tissues, such as liver and thyroid, are normally differentiated but they have still a limited power of regeneration in case the organ suffers any damage. The cells of the central nervous system in the adult have lost the capacity to divide with the disappearance of the ability to replace any worn out or damaged cell. The morphological changes in the cell during differentiation are less marked in the nucleus than in the cytoplasm. DNA is contained in the nucleus and carries heritable information in the sequence$_c$ of its four bases along its great length. The new cells and the parent cell must have the same DNA complement and hence the same chromosome content as the DNA of the cell is shared out among the individual chromosomes and is tightly coiled up within them. This orderly division of nuclear material is known as mitosis. The DNA doubles itself during interphase by replication in preparation for mitosis. The nuclear material becomes condensed at the outset of a division, into discrete individual chromosomes which form two identical halves called chromatids. The chromatids remain joined at the centromere. The nuclear membrane disappears, a spindle of hayline fibres is formed between the two chromosomes and the split chromosomes line up. The chromatids then separate and travel towards the poles of the spindle so that each new nucleus and the parent nucleus have identical genetic information. The cytoplasm divides with the formation of two new daughter cells, each containing the same DNA as the parent.

The chromosome complement is derived eqnally from the two parents in man and most of the higher animals. Their cells contain two numerically equal sets of chromosomes and the individual is said to be diploid. In man the diploid number is 46 and all Somatic cells have the same number of chromosomes. Of these 44 are autosomes and 2 sex chromosomes. In female the 2 sex chromosomes are called XX having the same shape and size and in the male they are XY which are clearly distinguishable from one another. Each human female has 22 pairs of autosomes alongwith an XX pair of sex chromosomes whereas males have 22 pairs of autosomes and an XY pair of sex chromosomes.

SEX DETERMINATION

Genes are carried in chromosomes and their segregation and independent assortment can be explained by the behaviour of the chromosomes during gamete formation. This is accomplished by a cellular process called meiosis as distinct from mitosis. The chromosomes are duplicated in mitosis before cell division, each daughter cell receiving a set of chromosomes identical with the parent cell. In meiosis, the maternally derived chromosomes pair with similar chromosomes (homologous) derived from the father, and line

up at metaphase. Members of each homologous pair are then transmitted to each daughter cell at the first reduction division resulting in the segregation of alleles. In the second reduction division, the chromatids are separated.

In mitosis, the equal distribution of chromatids to each daughter cell is independent of the arrangement of chromosomes at metaphase. The arrangement of the paternal and maternal chromosomes at metaphase of the first division in meiosis however, determ.¡ ies their particular distribution to each daughter cell. Their arrangement ..t metaphase is random hence their subsequent distribution or assortment is also random.

Sex determination is a genetic phenomenon depending on the constitution of the sex chromosomes. Each female gamete or ovum (primary oocyte) and each male gamete or spermatozoon contains only the haploid number of chromosomes and half the usual amount of DNA. Spermatogania contain 44 autosomes $+$ XY sex chromosomes, Y carrying male-determining genes. Oogonia contain 44 autosomes plus two identical sex chromosomes XX carrying female determining genes. When oocytes undergo reduction division, chromosome number in each ovum becomes half cont..ring 22 $+$ X similarly spermatocytes give rise to two kinds of secondary spermatocytes (becoming spermatozoa)—one type contains 22 $+$ X and the other 22 $+$ Y. The ovum may be fertilized by either kind of sperm resulting in following sex genotypes:

$$S_{,}\text{erm } X + \text{Ovum } X = \text{offspring } XX = \text{Female}$$
$$\text{Sperm } Y + \text{Ovum } X = \text{offspring } XY = \text{Male}$$

The genetic sex is thus dependent exclusively on the sperm and independent of the ovum (Fig. 1.4).
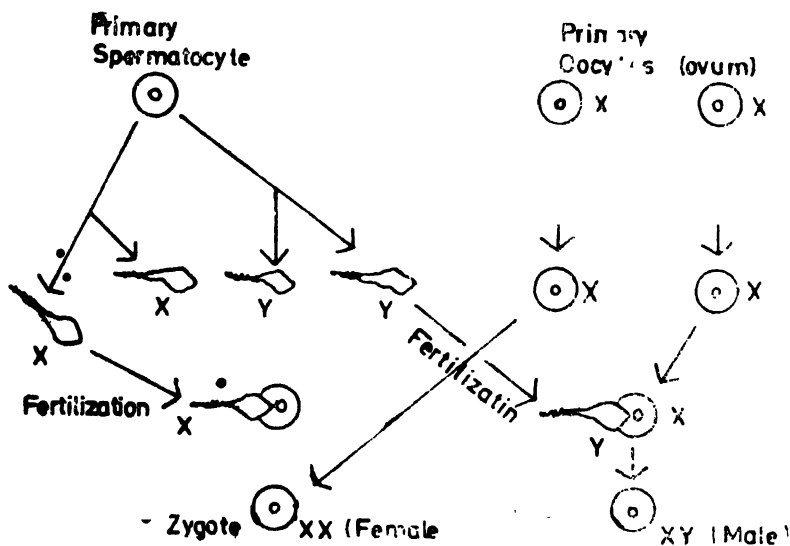

Fig. 1.4

The genetic sex usually determines the sex differentiation in the embryo.

CHROMOSOMAL MUTATIONS

The behaviour of chromosomes in mitosis and meiosis involve physical and chemical processes during which various kinds of abnormalities may happen. These are known as mutations, causing changes either in the structure of a particular chromosome or in the number of chromosomes. The changes in many cases are lethal, the zygote or embryo failing to survive.

The addition or removal of whole chromosomes results from the non-disjunction of homologous chromosomes during mitosis or meiosis. This may occur when the two chromatids of the replicated chromosome fail to separate at anaphase fast enough to get into the nuclei of daughter cells. One daughter cell thus receives one chromosome less and the other an extra. This type of mutation was first recognized and described for Drosophila. A number of human abnormalities have been found to be due to this. Mongolian idiocy is caused by the presenee of a very small extra chromosome produced by non-disjunction of number 21 of the 23 pairs of human chromosomes. A slant of the eye, a thick tongue, sagging mouth, unusual palm and sole prints, obesity, greatly retarded mental growth and shorter life are associated with changes in chromosome number. In certain organisms an occasional gynandromorph is produced, in which one side of the body is female and the other side male. This is due to the accidental loss of an X chromosome in one of the early cell divisions in the development of the embryo. Sometimes organisms have whole sets of extra chromosomes, a condition called polyploidy. A male with 69 chromosomes instead of the diploid number of 46 was reported in 1960. Chromosomes may break during the process of mitosis and meiosis, resulting in a variety of structural aberrations.

Classical genetics was developed on the concept that genes are arranged in a linear order on the chromosome, and that each has a distinct action. The action of genes is now known to produce continuous quantitative variations—a blending type of heredity.

A well-known case of multiple alleles in man is the blood type series A, B, AB and O. A and B are dominant over O, but when A and B are present in the same individual, neither gene is dominant and the individual is type AB. The phenotype and possible genotypes are:

Phenotype	Genotype
Type A	AA or AO
Type B	BB or BO
Type AB	AB
Type O	OO

The four classical blood groups depend on three genes named after the corresponding factor—A, B and O. Each person's blood group depends on the two genes which it receives from each parent. Thus:
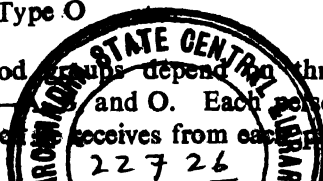
If a person receives

genes	A + A		B + B	A + B	O + O
or	A + O	or	B + O		
His blood group is	A		B	AB	O
His genotype is	AA or AO		BB or BO	AB	OO

Disputed paternity can be investigated on the basis of blood group classifications.

CHROMOSOME CHANGES IN ABORTIONS

Ten to fifteen per cent of all pregnancies terminate in spontaneous abortion before twenty weeks of gestation. Chromosome abnormality has been confirmed to be the cause. Klinefelter's syndrome is characterized by the presence of feminine stigmata in an apparent male with small testes, the sex chromosome pattern is usually XXY making a total of 47 in place of the normal 46 chromosomes. The sex chromatin test is positive, i.e., genetically female. In Turner's syndrome, growth and sexual development are retarded. Only one X chromosome is present with a total chromosome number of 45. The sex chromatin test is negative.

CHROMOSOMAL CHANGES WITH AGEING

Older persons have an increasing number of cells with a chromosome number of 45. The missing chromosome in the female is an X chromosome and the Y chromosome is missing in the male. In continually dividing cells chromosome aberrations, including loss of chromosomes, would be expected with ageing. Loss of one X in the female or the Y in the male would not be lethal. The occurrence of these cells may be a reflection of a much larger amount of aberration from which cells do not survive. The possible relevance of the finding to the understanding of the ageing process is evident.

CHROMOSOME CHANGES IN CANCER

In many cases of a major form of leukemia, so-called chronic myeloid (or granulocytic) leukemia, a chromosomal aberration in the form of a deletion of part of the long arm of one of the four small acrocentric chromosomes (either 21 or 22), has been found. The abnormality is confined to blood cells.

It is certain that the chromosome 21 or 22 is implicated in leukemia as in Down's syndrome. It has been, however, suggested (based on several observations) that the same chromosome is involved, in different ways, in both leukemia and Down's syndrome. Leukocyte alkaline phosphatase is higher than normal in these patients and lower than normal in those with myeloid leukemia. Chronic myeloid leukemia in

many cases is caused by the deletion of chromosome 21 in the leukocytes line. This is perhaps acquired, not inherited—exposure to X-rays may be one cause. The biochemical disorders in conditions as Fanconi's anaemia and Bloom's disease, which are inherited as autosomal recessive conditions, may be due to either the chromosomes being rendered more vulnerable to the effects of chromosome—breaking factors in the environment, such as viruses, or the mechanisms normally responsible for chromosome repair becoming faulty. Malignancy, particularly leukemia, occurs commonly in patients with Fanconi's anaemia or Bloom's disease, and may be a result of chromosomal breakage.

The chromosomal basis of certain congenital malformations, sex anomalies, behavioural abnormalities, spontaneous abortions, and neoplastic diseases has been elucidated. The chromosomal changes with ageing have also been discovered. Chromosomal breakage leading to structural abnormalities, such as deletions, translocations isochromosomes and inversions have been identified.

INBORN ERRORS OF METABOLISM

The proteins are the products of gene action. Haemoglobin, which is not an enzyme in the strict sense, has an important function in oxygen transport. The functions of the hepatoglobins, transferrins, and gamma globulins, all of which are non-enzymatic serum proteins, are partly understood. But some of the proteins specified by genes are enzymes. A mutation in the gene that determines a given enzyme may produce a disorder named by Garrod as inborn errors of metabolism.

Alkaptonuria was the basis of Garrod's concept of inborn errors of metabolism. An enzyme homogentisic acid oxidase (homogentisicase) is involved in the metabolism homogentisic acid giving rise to the defect (Fig. 1.4c). Large amounts of homogentisic acid are excreted in the urine and turns black in alkaline urine or exposure to light. Aggregates of homogentisic acid accumulate in the body, attached to the collagen of cartilage and other connective tissues. The cartilage of the ears and the sclera are stained black, a condition called ochronosis. The accumulation of the acid in the joints, such as those of the spine leads to arthritis. Alkaptonuria results from a genetic enzyme block in which the phenotypic features are caused by the accumulation of excess substances just proximal to the block (Fig. 1.4b). Phenylketonuria (PKU), like alkaptonuria and albinism, is a genetic defect in aromatic amino acid metabolism (Fig. 1.5a). In albinism the genetic block involves a step between the amino acid tyrosine and the pigment melanin. In phenylketonuria the defect is in the enzyme involved in the conversion of phenylalanine to tyrosine. The pigmentation of the affected person is lighter than normal but the affected person is not complete albino since tyrosine is available in the diet.

Certain metabolic products of phenylalanine formed through alternative pathways give rise to untoward effects on brain metabolism resulting

in severe mental retardation. Some of these alternative metabolites of phenylalanine, especially phenylpyruvic acid, are excreted in the urine forming one of the basis for diagnosis of the disorder. The difference in phenotype of these three diseases—alkaptonuria, PKU and albinism—despite the fact that they involve closely related metabolic steps, is noteworthy.
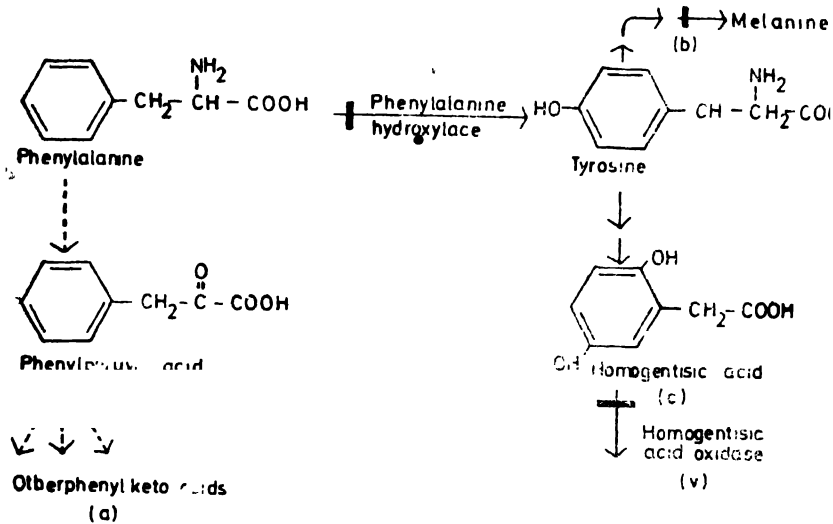

Fig. 1.5

All inborn errors of metabolism are essentially inherited as recessives—the clinical disorder is present only in the homoz· ote. In PKU the phenotypically normal but genetically heterozygous parents of affected persons tend to have higher levels of blood phenylalanine which last longer than normal when a standard dose of this amino acid is administered—a test known as phenylalanine tolerance test. The enzyme defect can be conveniently demonstrated in the circulating erythrocytes. The heterozygote has an enzyme level intermediate between that of the two homozygotes, the normal and the affected.

IMMUNOGENETICS: BLOOD GROUPS

The blood groups alongwith the haemoglobins have contributed largely to the formulation of the principles of human genetics. Blood groups are also of great significance in medicine. The blood groups are genetically determined antigens of the erythrocytes. The different antigens on the red cells are identified by means of antibodies—proteins in serum that combine with the antigens producing such effects as agglutination of these cells. Some of the antibodies occur naturally; for example, in the ABO blood group system, type A persons have in their serum antibody against

type B cells; type B persons have antibody against type A cells; and type O persons have antibody against both type A and type B cells. Blood groups are of great genetic significance providing some of the clearest early examples of simple Mendelian inheritance.

The importance of blood groups to medicine lies in at least three areas: (1) blood transfusion with no resulting complications requires recognition and understanding of the genetic differences; (2) the proper management and prevention of the ill-effects of maternofetal incompatibility (the Rhesus—Rh problems of pregnancy); (3) medicolegal applications in disputed parentage. A man is excluded as father if he and the mother both lack the antigen that the child possesses. He is also excluded if the child fails to show an antigen he must transmit. An AB man cannot have an O child, nor can an M man have an N child.

CAUSES OF BIRTH DEFECTS

A relatively simple genetic defect may give rise to several congenital malformations—autosomal recessive acheiropody (absence of hands and feet). A relatively simple environmental or extrinsic cause may cause a few congenital malformation. Rubella (German measles) occurring in the first twelve weeks pregnancy causes malformations of the heart, eyes and other organs. Consumption of thalidomide (a tranquilizer and sedative) during early pregnancy may lead to phocomelia (seal limbs). The majority of congenital malformations—cleft palate, harelip, clubfoot, anencephaly and congenital heart malformation, may be the result of a collaboration of genetic and environmental factors.

Despite our present knowledge of human genetics, most scientists in the field consider the insight obtained as vague. Seven Nobel Prizes in physiology and medicine have been awarded to workers in the area of genetics:

1933 Thomas Hunt Morgan (1866–1945) for his research on the nature of the gene.

1946 Hermann Joseph Muller (1890–1967), for his discovery of the induction of mutation by X-ray.

1958 George Wells Beadle (1903–) and Edward Lawrie Tatum (1909–) for contributions in biochemical genetics and Joshua Lederberg (1925–), for discovery of sexual recombination in bacteria.

1959 Arthur Kornberg (1918–) and Severo Ochoa (1905–) for studies of the chemistry of DNA and RNA.

1961 James D. Watson (1928–), Francis H.C. Crick, (1916–) and Maurice H.F. Wilkins (1916–), for elucidation of the intimate structure of DNA.

1965 Francois Jacob (1920–), Andre Lwoff (1912–), and Jacques Monod (1910–76), for their discoveries concerning genetic control of enzyme and virus synthesis.

1968 Marshall W. Nirenberg (1927–), H. G. Khorana (1922–), and Robert W. Holley (1922–), for cracking the *genetic code* and elucidating the means by which a gene determines the sequence of amino acids in a protein.

Further Reading

David F. Horrobin, *Medical Physiology and Biochemistry* (London: Edward Arnold, 1968).

Samson Wright, *Applied Physiology* (London: ELBS, 1971).

Bailey, *Textbook of Histology* (London: Williams & Wilkins Co., 1971).

Bell and Davidson, *Textbook of Physiology and Biochemistry* (London: ELBS, 1972).

G. N. Ramachandran, Ed., *Aspects of Protein Structure* (London: Academic Press, 1963).

H. L. Roman and A.S. Sussman, Ed., *Topics in the Study of Life* (London: Harper & Row, 1970).

V.A. McKusick, *Human Genetics* (Delhi: Prentice-Hall of India, 1972).

P. B. Weisz, *The Science of Biology* (New York: McGraw-Hill, 1971).

R.B. Platt and G.K. Reid, *Bioscience* (New York: Reinhold Book Corporation, 1968).

TWO

Organization of the Human Body

Morphogenesis

Life began through a progressive series of chemical synthesis reactions
that raised the organization of inanimate matter to successively higher
levels. Atoms first formed simple compounds, which transformed into
more complex ones and the most complex of them eventually became
organized as living cells.

Normal development begins with a sperm-fertilized egg, a single cell in
which fusion of a male with a female pronucleus has occurred. This cell
divides into daughter cells, which continue to divide and adhere, thereby
forming a multicellular mass. Earlier cell divisions are primarily cleavages
of the original egg-cell substance but the process soon becomes associated
with actual growth with the rapid increase in mass.

The accumulating cells have little difference during the early divisions
and begin to arrange in three distinct layers—the ectoderm (outer),
mesoderm (middle) and endoderm (inner). These are referred to as the
three fundamental germ layers. The cells of each of the three germ
layers divide, differentiate and group themselves into specialized tissues,
which in turn are organized into organs and organ systems. The outer
epithelia and the nervous system develop from the ectoderm; the lungs,
the gut epithelium and its derivatives from the endoderm; the blood,
skeleton and muscles, and also the organs of excretion and reproduction
from the mesoderm.

The adult tissues are not composed of cells only. At a very early
stage of differentiation, the cells become separated from each other by the
formation of intracellular substance which may result from cellular
secretion or may represent actual modification of cellular protoplasm.
Each tissue therefore, is an aggregate of cells with its own characteristic
intracellular substance, called a matrix. When the intracellular substance
is small in amount, the cells are in close contact with one another in the

tissues, such as in the epithelium, muscle. With large amounts of intracellular substance, the tissues may contain highly differentiated elements of important physiological significance. Such examples are bone, cartilege, connective tissue and blood.

The adult tissues are classified into four main groups: (1) epithelial tissue, (2) connective tissue with its derivatives, blood and lymph, (3) muscle tissue, (4) nerve tissue. The epithelial and connective tissues are regarded as more elementary tissues. Muscle and nerve constitute the most highly specialized tissues. Blood is a unique tissue, closely related to the connective tissues, in which cells are suspended and move freely in a fluid intracellular substance.

The body tissues contain large proportions of fluid. The vital physiological reactions depend upon fluid media and the fluid-filled cavities and the fluid-bathed surface membranes are indispensable in the organization, to enable the tissues to serve their functions.
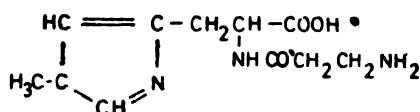
Chemistry of the Tissues

MUSCLE TISSUE

Muscle tissue are classified into three groups: striped or striated (voluntary); smooth or nonstriated (involuntary) and the cardiac muscle. The skeletal muscles of the body belonging to the first group, constitute almost half of the total body weight. The second group occurs in the walls of the bladder, skin, arteries and veins. The third forms the main part of the wall of the heart.

Muscle is composed of 75 per cent water and 25 per cent solids. Proteins constitute 20 per cent of the solids, rest of which includes carbohydrates, salts, and nitrogenous compounds, called extractives. Creatine, phosphocreatine, purine bases, uric acid, lactic acid, adenylic acid and its derivatives (ADP and ATP), carnosine and anserine belong to the latter. Carnosine and anserine are peptides and little is known about their physiological role.

$$HC = C-CH_2-CH-COOH$$
$$HN \diagdown_{CH} \diagup^N \quad NH-COCH_2CH_2NH_2$$
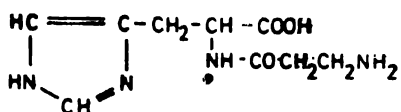
Carno.

(dipeptide of histidine and β-alanine)

$$HC = C-CH_2CH-COOH$$
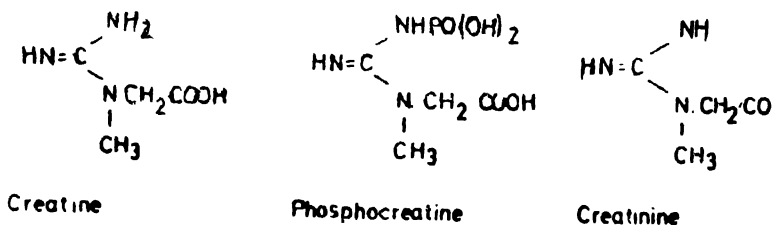$$H_3C-C \diagdown_{CH} \diagup^N \quad NH COCH_2 CH_2 NH_2$$

Anserine

(dipeptide of methyhistidine and β-alanine)

Creatine

Largely, in the form of phosphocreatine, creatine is found in muscle, brain and blood. It functions in muscular contraction and in carbohydrate
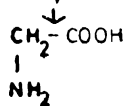
metabolism. Creatine appears to be confined to vertebrates. Arginine plays a similar role in invertebrates. The anhydride of creatine, creatinine, is found in the urine of vertebrates.
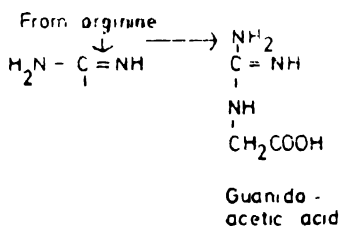
$$HN=C\overset{NH_2}{\underset{\underset{CH_3}{|}}{\overset{\diagup}{\underset{N\,CH_2COOH}{\diagdown}}}}$$

Creatine

$$HN=C\overset{NHPO(OH)_2}{\underset{\underset{CH_3}{|}}{\overset{\diagup}{\underset{N\,CH_2\,COOH}{\diagdown}}}}$$

Phosphocreatine

$$HN=C\overset{NH}{\underset{\underset{CH_3}{|}}{\overset{\diagup}{\underset{N.CH_2CO}{\diagdown}}}}$$

Creatinine

Biosynthesis of Creatine

Isotopic creatine and creatinine are formed when rats are fed with isotopic guanidoacetic acid (glycocyamine). The biosynthesis involves two steps: production of guanidoacetic acid and methylation of the compound produced.
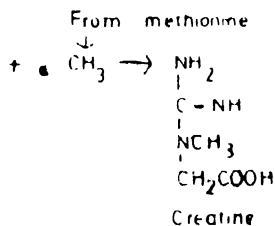
From proteins
$$\underset{NH_2}{\overset{CH_2-COOH}{|}}$$

From arginine
$$H_2N-\underset{|}{\overset{|}{C}}=NH \quad \dashrightarrow \quad \underset{\underset{CH_2COOH}{|}}{\overset{\underset{NH}{|}}{\overset{C=NH}{\overset{NH_2}{|}}}}$$

Guanido-acetic acid

$$+ \quad CH_3 \longrightarrow \underset{\underset{CH_2COOH}{|}}{\overset{\underset{NCH_3}{|}}{\overset{C-NH}{\overset{NH_2}{|}}}}$$

From methionine

Creatine

The methyl group of methionine is utilized for the formation of muscle creatine, but neither creatine nor sarcosine can provide its methyl group for the conversion of homocystine to methionine.

All of the nitrogen in the amidine group of creatine is recovered as NH_3 and the remaining nitrogen in the form of sarcosine.

$$\underset{\underset{CH_2COOH}{|}}{\overset{\underset{N\,.CH_3}{|}}{\overset{C=NH}{\overset{NH_2}{|}}}} \xrightarrow[(2H_2O)]{Ba(OH)_2} 2NH_3 + CO_2 + \underset{\underset{CH_2\,COOH}{|}}{HN} + CH_3$$

Creatine   Sarcosine
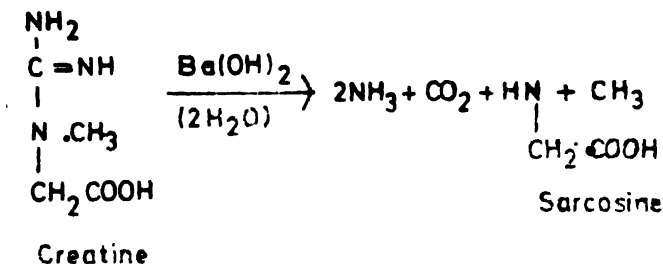
Creatine is not excreted by normal adult male. But it appears in urine in starvation, fever and when muscle atrophies. Creatinuria occurs intermittently in adult female and also during pregnancy. Until puberty, children of both sexes excrete creatine as well as creatinine.

Proteins in Muscles
Distribution of protein fractions in white muscle is:

Protein	Per cent of total protein
Myogen	9
Globulin X	18
Myosin	57
Stroma	16

Myogen is the so-called albumin fraction of muscle. It contains a mixture of several proteins—myogen A and myogen B have been isolated in crystalline form. About 16 per cent of myogen fraction constitute the enzymes, isomerase, aldolase, triosephosphate dehydrogenase and phosphorylase. Little is known about globulin X. Stroma is perhaps the source of action, which in combination with myosin, produces actomysin, the contractile protein.

Muscle also contains, in the satcoplasm of the cell, the respiratory pigments, myoglobin and cytochrome which function in the transport of oxygen from the blood to the oxidizing systems. Muscle contains only small amount of lipid. Carbohydrate is present mostly as glycogen to the extent of 0.5 to 1.0 per cent. One of the most important compounds in muscle is adenosine triphosphate (ATP) which is a high-energy compound. It is the energy released by the breakdown of ATP that is used in the performance of muscular work.

Chemistry of the Contractile Protein
Myosin is a fibrous protein with a molecular weight 4,70,000. It is a rod-shaped molecule consisting of two identical very long polypeptide chains, each containing about 1800 amino acids. Each chain is in the α-helical configuration and the two chains are wound round each other to form a double helical structure. Both chains are folded into a globular head at one end of the molecule. Myosin molecule is split into two portions by the action of trypsin—the heavy fragment, called heavy meromyosin containing the globular head and the light fragment, light meromyosin. Myosin is itself or is inseparably associated with the enzyme adenosine triphosphatase (ATPase)—the enzyme that catalyzes the breakdown of ATP to ADP and its activity is located in the globular head of the myosin molecule.

Szent-Gyorgyi in 1941 discovered actin, existing in two forms, G-actin (globular actin) and F-actin (fibrous actin). G-actin is globular in shape consisting of a single polypeptide chain with a molecular weight of 46,000. Each molecule can bind one calcium ion very strongly and has a high affinity for ATP. ATP and G-actin binding results in polymerization to F-actin, one molecule of ATP being split to ADP and inorganic phosphate. ADP so formed remains attached to the G-actin units. F-actin is composed of two long strands of G-actin units coiled round each other.

In solution, myosin binds actin at two specific sites, probably in the

heads, to form actomyosin with a large increase in viscosity. Actomyosin dissociates into actin and myosin with a sharp drop in viscosity in presence of ATP and magnesium ions. At the same time, myosin splits ATP into ADP and inorganic phosphate. With the hydrolysis of all the ATP, the actomyosin complex reforms. The splitting of the actomyosin complex by ATP appears to be similar to the process of detaching side chains of the myosin from actin in an intact muscle fibre.
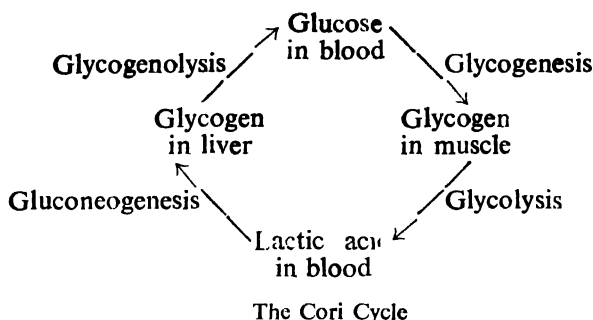
ATP is probably the immediate source of energy for muscular contraction, the ultimate source being carbohydrate or fat. The chemical process during muscle contraction probably involves the sequence of events—muscle at rest contains G-actomyosin which reacts with ATP to form F-actin. F-actin polymerizes and combines with myosin to form F-actomyosin, which then contracts and liberates ADP. ADP is reconverted to ATP by the action of phosphocreatine or other energy-rich phosphate compounds during the relaxation of muscle. The second step is the breakdown of carbohydrate in muscle by the process of glycolysis to yield pyruvate. The carbohydrate may be both stored as glycogen and glucose from the blood, both of which are converted during early stages of glycolysis into glucose-6-phosphate. ATP is synthesized in the process. Some of the pyruvate is reduced to lactate when the oxygen supply is not adequate. Both pyruvate and lactate are oxidized by molecular oxygen to yield carbon dioxide and water through the operation of citric acid cycle (tricarboxylic acid cycle) constituting the final stage in muscle for their aerobic disposal.

Fatty acids, especially in cardiac muscle, also provide much of the energy utilized in muscular work. Fat is mobilized during muscular exercise, directly as non-esterified fatty acids, which are conveyed in the blood, loosely bound to serum albumin, to the muscles and oxidized through citric acid cycle. Carbohydrate is the major fuel during muscular activity and mammalian skeletal muscle uses fatty acids and acetoacetate as fuel in the resting state.

Repeated muscle contractions may lead to the production of lactic acid in muscle. It may happen when the rate of glycolysis is very rapid or when the available oxygen is insufficient for the immediate oxidation of pyruvate to carbon dioxide and water. Part of the pyruvate is then converted into lactate. When the concentration of lactic acid is high in muscle, large proportion of it diffuses into the blood, which carries it to the liver for conversion into glycogen through Cori cycle.

The Cori Cycle

Muscle glycogen is broken down during sudden vigorous muscular exercise anaerobically to lactic acid which diffuses into the blood. The liver can convert the lactic acid back to glycogen - by reversal of the glycolytic pathway. The liver glycogen in turn, can break down to free glucose which is carried by the blood to the muscles for replenishing muscle glycogen.

Glucose
in blood

Glycogenolysis   Glycogenesis

Glycogen   Glycogen
in liver   in muscle

Gluconeogenesis   Glycolysis

Lactic acid
in blood

The Cori Cycle

Lactic acid levels upto 200 mg per 100 ml blood can be tolerated by a highly trained athlete (normal range is 5-20 mg per 100 ml). This is equivalent to the conversion of 100 g muscle glycogen to lactate.

The presence of thiamine pyrophosphate (TPP) derived from vitamin B_1 thiamine, is necessary for the oxidation of pyruvate by the citric acid cycle. In vitamin B_1 deficiency (beri-beri) even mild forms of exercise lead to an increase in the blood pyruvate (normal fasting average is 0.76 mg per 100 ml blood) due to lack of sufficient cocarboxylase (TPP) to bring about its rapid oxidation. This phenomenon has been used as a basis for assessing thiamine deficiency.

Muscular exercise involves circulatory and respiratory adjustments. The pulse rate blood pressure rise more slowly in persons in good training compared to untrained persons. The oxygen consumption increases during muscular exercise and respiration is quickened.

Hormonal changes in exercise are reflected in increased activity of the adrenal cortex (fall of upto 80 per cent in the circulating eosinophils and manifold increase in the output of 17-hydroxysteroids in urine), raised serum growth hormone (HGH) and fall in plasma insulin. The HGH probably initiates and maintains the mobilization of depot fat during exercise. The fall in plasma insulin in exercise allows the mobilization of fat and prevents the blood glucose falling too low.

NERVE TISSUE

The nerve tissue includes brain, spinal cord, peripheral nerves, ganglia and plexuses. Its importance lies in its ability to respond to stimuli and to conduct impulses. The gray matter of the brain may contain 80 per cent or more of water. 50 per cent of the solids come from the proteins of which collagen and neurokeratin are most abundant in the nerve tissues. According to Block, the neurokeratin may be the protein in the neurofibrils, the filaments in the nerve cells, and their axons.

Relatively small quantities of alkaline phosphates, phosphocreatine, adenosine triphosphate, hexosephosphate, chlorides, carbohydrates, extractive (creatine etc.), and inositol are also present. Besides proteins, lipids constitute the largest and the most characteristic materials in the

nerve fibres. The lipids include lecithin, cephalin, sphingomyelin, cerebrosides and sterols (particularly cholesterol), in addition to true fats. Niemann-Pick disease found in childhood, is characterized by an increase in the sphingomyelin of the brain. The cephalin in brain has been shown by Folch to consist of a mixture of three different phosphatides—one contains ethanolamine, another serine and the third inositol.

When a peripheral nerve is cut, the portion of the nerve distal to the point of section soon loses its ability to transmit nerve impulse. This is associated with histological changes known as Wallerian degeneration. The lipid-rich myelin which surrounds the axon of the nerve, is completely destroyed with the degeneration of the nerve.

Metabolism of Nerves

The neuron is the unit of the nervous system. It consists of the cell, the dendrites, and the axon. The axon (axis-cylinder) is the central core of a nerve fibre. The respiratory quotient (RQ) of brain is very nearly 1 and the oxygen utilized is equivalent to the amount of glucose which disappears (4.9 mg per 100 gm per min), as evident from a comparison between arterio-venous differences in oxygen, carbon dioxide and glucose. Glucose appears to be almost the exclusive source of energy for the brain tissues, glycogen content being too low, about 0.1 per cent.

The brain and all the nerve tissues is characterized by a high lipid content. The human brain is composed of:

        Water   = 76.9 per cent (per cent fresh weight)
        Protein   = 37.7 per cent (per cent dry weight)
        Lipid   = 54.4 per cent
        Extractives = 7.9 per cent

The oxygen consumption of the brain is reduced in conditions in which the consciousness is depressed—insulin hypoglycaemia reduces it by 20 per cent, diabetic coma by 50 per cent, and surgical anaesthesia by 40 per cent whereas sleep reduces it only by 3 per cent. Oxygen consumption of the brain does not increase due to mental effort.

Rapid growth of the brain in the foetus and infant is accompanied by rapid synthesis of new proteins. The synthesis slows down gradually and it is almost the same as in other tissues in adult life. The water soluble proteins of brain tissue are similar to those tissues having a comparable turnover. The brain contains, however, two special types of proteins—the lipoproteins and the phosphoproteins. The lipoproteins of the myelin sheath contain protein with such a low turnover that it can be regarded as metabolically inert. The phosphoproteins of nerve cells contain phosphorus with a very rapid turnover which increases with electrical stimulation.

When the nerve is stimulated ammonia is produced. The significance of the production of ammonia is not clear, may be, it is due to the

presence of two substances in the brain tissue—the adenylic acid and glutamine. The former may lose ammonia yielding inosinic acid and the latter breaks down to glutamic acid and ammonia.

$$CONH_2.CH_2.CH_2.CHNH_2.COOH \rightarrow$$

Glutamine

$$NH_3 + HOOC.CH_2.CH_2.CHNH_2.COOH$$

Ammonia   Glutamic acid
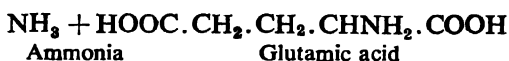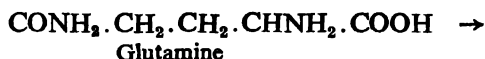
Glutamine is probably formed first by the combination of glutamic acid and ammonia by the reverse process.

Nervous tissue is relatively isolated from the rest of the body. This is due to the difficulty with which many substances such as amino acids, pass into the nervous tissue from the blood and to the very limited ability of the tissue to store carbohydrate in the form of glycogen. The brain's dependence on glucose makes it very sensitive to hypoglycaemia. About 25 per cent of the glucose is utilized by the brain for energy production through the tricarboxylic acid pathway: remaining 75 per cent is used for the formation of amino acids, chiefly glutamic acid and aspartic acid, which are used partly for protein synthesis but mainly for oxidation by pathways which exist in the nerve cell for the production of energy. The free amino acids concentration in nerve tissue is eight times higher than the blood plasma. Fifteen per cent of the pool of amino acids consists of γ-amino butyric acid (GABA) which is formed by the decarboxylation of glutamic acid.

Cyclic AMP appears to play a key role in brain function. The concentration of cyclic AMP in brain is increased by catecholamines and histamine, the largest responses are found in the cerebellar tissue. The brain tissue, among all mammalian tissue, has the highest capability of synthesizing cyclic AMP Cyclic AMP-dependent protein phosphokinases have been demonstrated in brain. Cyclic AMP level is raised by electroconvulsive shock treatment and anoxia. Cyclic AMP content in urine is lowered in cases of severe depression and elevated in maniac patients. Cyclic AMP appears to be involved in the release of acetylcholine from nerve endings under the influence of adrenaline.

The nerve can produce lactic acid from carbohydrate in the absence of oxygen. The lactic acid is oxidized very slowly. It is still not certain as to whether the stimulation of a nerve involves a glycogen-lactic acid metabolism at all comparable to what takes place in the muscle.

### The Membrane Theory of Conduction

It is assumed that the nerve is surrounded by a polarized membrane, the outside of which carries a positive charge and the inside a negative charge. This is due to the fact that the concentration of potassium ions is higher inside than outside the membrane. The basis for the membrane theory of nerve conduction is found in this selective permeability to potassium

ions, and the leakage of potassium ions during nerve activity. When a stimulus is applied to the surface membrane, a reversal of charge takes place and the outside surface becomes negative to an adjacent point on the membrane. In this way a flow of current results which stimulates an adjacent point. An exchange of potassium ions takes place across the nerve membrane even during the resting stage establishing a dynamic concentration gradient with respect to this ion, as well as to sodium ions, which are more concentrated outside than inside the membrane. The flow of current in an isolated nerve after a suitable stimulus has been applied, can be seen and measured by cathode ray oscilloscope. The current which is produced is known as the action potential.

Vitamins

A number of vitamins are required for the prevention of degenerative changes in the central nervous system which has received the utmost attention so far. The vitamins are almost exclusively the B-Complex and Vitamin B_1 or thiamine. The brain oxidizes carbohydrate almost exclusively—for which insulin is not required—unlike other organs like muscle, heart, kidney and liver. Vitamin B_1 deficiency gives rise to beri-beri which is accompanied by an increase in the amount of pyruvate in the brain and a decrease in oxygen consumption. The oxidation of glucose in the brain may be according to the following pathway:

Glucose → Lactic acid → Pyruvic acid → Carbon dioxide
Vitamin B_1, CoA and α-lipoic acid are involved in the change of pyruvic acid to acetic acid and CO_2 through a process of oxidative decarboxylation:

$$CH_3CO.COOH \xrightarrow[+O]{-CO_2} CH_3COOH + CO_2$$
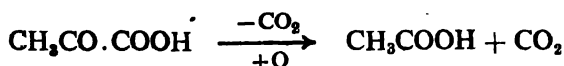
EPITHELIAL TISSUE

The epithelial tissue is found in the covering of the surface of the body (skin), in the lining of the respiratory tract, as an essential part of the glandular organs, etc. The characteristic substance present in this tissue is the albuminoid keratin. Among proteins it is the most resistant to chemical action. It is insoluble in any solvents which dissolve other proteins and it is not attacked by gastric or pancreatic juice. From 16 to 21 per cent of cystine is present in human hair. By grinding exhaustively keratin becomes more digestible. Male hair appears to contain more cystine than female hair and dark hair contains more cystine than light hair. The colour of the normal skin is due to several pigments—melanin, carotene, reduced haemoglobin and oxyhaemoglobin.

CONNECTIVE TISSUE

Collagen (white fibrous tissue), elastin (yellow elastic tissue), chondromucoid

(mucoprotein of cartilage) and the variety of substances—the ground substances of the cell—constitute the connective tissue.

BONE (OSSEOUS TISSUE)

Normal mature bone contains nearly 50 per cent its weight of water and at times as much as 24 per cent of fat. The chief inorganic constituents are calcium, phosphate, and carbonate. Organic constituents include citric acid.

Further Reading

Bailey, *Textbook of Histology* (London: Williams & Wilkins, 1971).

Paul B. Weisz, *The Science of Biology* (New York: McGraw-Hill, 1971).

R.B. Platt and G.K. Reid, *Bioscience* (New York: Reinhold, 1968).

B. Harrow and A. Mazur, *Textbook of Biochemistry* (New York: Saunders, 1958).

G.H. Bell, .'.N. Davidson and D.E. Smith, *Textbook of Physiology and Biochemistry* (London: ELBS, 1972).

Blood, Lymph and Other Body Fluids

Introduction

Cells are in steady state relative to their immediate surroundings. In animals, other cells and, in most instances, the body fluids, blood and lymph constitute these surroundings. These fill up all the spaces between cells and cell layers. Cells and tissues thus reflect the conditions prevailing in blood and lymph, and vice versa.

Blood may be considered as a tissue consisting of free cells (corpuscles) and a fluid intracellular substance called plasma. Blood is related to the connective tissues both structurally and genetically. In the reticular connective tissues of blood forming organs blood cells develop and enter the blood stream in a fully formed condition.

The attributes of the body fluids are controlled primarily by three systems. The circulatory system regulates the physical properties of the fluid it carries, such as pressure, distribution, and the rate of flow. The chemical properties of the body fluids govern the breathing and excretory systems. Both systems monitor and adjust continuously the composition of the body fluids as well as control the exchange of materials between body fluids and the external environment. In the process they also determine what is or is not a waste product.

Unicellular organisms like the amoeba obtain food and dispose of waste products through processes of diffusion with the aqueous media in which the organism exist. The organisms thrive when the diffusion processes can maintain a rather definite composition of the fluids within the cell. This process is known as *homoeostasis*. Accumulation of waste products, depletion of food substances or other causes bring about changes in the composition of the surrounding fluid, prevent the maintenance of proper composition of the intracellular fluids with the result that the organism ceases to function.

LYMPH

The internal fluid in ancestral animals was probably little different from sea water, and the modern animals have inherited a form of sea water which may be called lymph, as the universal internal medium of their bodies.

Lymph functions chiefly in maintaining water, salt, pH, and osmotic equilibria between the interior and exterior of cells. Lymph also provides a medium for the diffusion and transport of foodstuffs, respiratory gases, waste materials, and in some cases hormones, and any other substances that pass from one body region to another. The body fluids in many animals are kept in motion by means of specialized flow channels and pumping organs, or circulatory systems. Any body fluid confined partly or wholly in circulatory channels is blood.

BLOOD

Blood channels in different animals are either open or closed. In an open system blood and lymph are essentially indistinguishable and both the body fluids contain the same components. Most materials in blood pass through vessel walls readily in a closed system, blood proteins and blood cells, however, cannot largely do so. All blood components other than the cells constitute what is called plasma and the presence of such a medium between blood cells makes blood a tissue.

The structural components of mammalian blood are not all true cells, they are therefore designated as the formed elements. They include red corpuscles (erythrocytes), the white blood cells (leukocytes) and the blood platelets (thrombocytes). The total quantity of blood (formed elements and plasma) accounts for about 8 per cent of the body weight. There are some 5 or 6 litres of blood in a man weighing 150 pounds.

Hematocrit
The percentage of formed elements present in total blood can be determined by centrifuging the blood in a graduated tube. The percentage of formed elements is known as the hematocrit.

The number of erythrocytes per cubic millimeter of blood can be known by diluting a known quantity of blood with a known amount of isotonic fluid in a special pipette and then counting the number in a drop of the mixture placed in the chamber of a hemocytometer slide. From a count of the erythrocytes in a given number of squares, the total number of erythrocytes per cubic millimeter can be readily calculated. This is known as total erythrocyte count, which is of particular importance in studies of different types of anaemia. Dried blood smears are stained with particular types of compound dyes (Wright's stain) to give differentiation of neutrophilic, eosinophilic and basophilic components leukocytes. The determination of different leukocyte percentages by this method is known as the *differential count.*

BLOOD PLASMA

The main ingredient of plasma is water derived from food and metabolic water which is exported from cells to the body fluids. The plasma is a histologically homogeneous, slightly alkaline fluid. It contains globulins, albumins and inorganic salts—chiefly chloride, bicarbonate and phosphate of sodium. Calcium is present in a remarkable constant quantity (1 mg per 10 ml of blood). The plasma constitutes 55 per cent of the total quantity of blood and the formed elements account for 45 per cent. This means that the hematocrit value for normal blood is 45. The proportions undergo alterations in a number of pathological conditions—in microcytic anaemia there is a reduction in size and number of erythrocytes, which lowers the hematocrit value.

The supply of water is carefully adjusted by elimination of excess amounts through the breathing and excretory systems. This is how the total water content of the body, hence the blood volume, is maintained constant. The water of plasma influences blood pressure, and it is the transport vehicle of blood cells and numerous dissolved materials.

The dissolved components are of two general types: One comprises substances such as a number of foods, hormones, certain waste products (urea) and many other compounds that fluctuate more or less widely in concentration, depending on body activity. The second contains, apart from water, mineral ions, foods such as glucose, and a number of other compounds that are either nutrients in transit to tissue cells or waste products in transit to the excretory organs, normally maintained at constant concentrations by a balance between supply and removal. Supplies are obtained from tissue cells via lymph and the alimentary system, particularly the gut and the liver. Removal can involve the liver storage, elimination through the excretory system (kidneys, lungs, intestines, skin), or passage to lymph and later absorption by tissue cells. In each case too high or too low a concentration of a 'given substance in blood is the critical stimulus for its own removal or replenishment. For example, a moderately high blood glucose level stimulates liver cells to reduce the concentration by storing the excess as glycogen. A still higher level stimulates storage not only in liver but also in muscle and skin; and a very high concentration leads to glucose excretion from the kidneys.

The mineral ions in plasma are of the same types as those in lymph and in the interior of cells in general. These ions are responsible for the salt pH, and osmotic balance between plasma, lymph, and tissue cells. The intake of much salt water would raise the mineral concentration of blood and the tissues would lose water osmotically, and would become dehydrated. Conversely, intake of too much plain water lead to a decline in ion concentration in blood and the tissues would gain water. Temporary fluctuations of this sort with accompanying pH changes can occur frequently. Excretory organs, which eliminate any excess material, impose fairly narrow limits. From hour to hour, therefore, blood has

constant osmotic pressure and pH.   The pH in human blood is normallly 7.3 or 7.4.

Plasma differs from lymph by having an appreciable concentration of blood proteins kept at constant level.   In vertebrates these proteins are manufactured largely in the liver.   They generally leave the circulation only in very limited amounts and serve in a variety of nonnutritional roles.   All these proteins, like the mineral ions, contribute to maintaining the osmotic pressure and pH of blood.   Albumins are of particular importance in the osmotic regulation.

When blood is exposed to the air or when blood vessels are injured, one of the globulins of the plasma (fibrinogen) precipitates out as a network of delicate filaments, the fibrin, leaving a clear yellowish fluid, the serum.   The blood cells become entangled in the fibrin network and a clot is formed.   The clot acts as a plug preventing further haemorrhage.   Many of the proteins are active enzymatically, such as prothrombin, a clotting factor.   A clot may be torn off by the blood stream to circulate in the blood vessels (embolus), which may block the blood supply of vital organs—a condition beset with great danger to the individual.

Globulins are the basis of differences in the blood types.   Some of the globulins also serve as defensive antibodies, which destroy or render harmless infective agents such as bacteria.   Animals can thereby become immune, an important aspect of steady state control that is ultimately a consequence of protein specificities.   Apart from its protein content, plasma in many animals differs from lymph in another respect also—the presence of respiratory pigments.   In good many animals the pigments, however, occur not in plasma but in blood cells.

The plasma is the fundamental substance mediating all nutrition.   The nutritive substances derived from the alimentary can t, the waste product from the tissues and the secretions of the various endocrine glands, are dissolved in the plasma.   Even the oxygen which is bound by the red blood cells is first dissolved in the plasma before reaching the cells.   The greater constancy of the constituents of the plasma makes it distinct from the tissue fluids.   The plasma proteins of the blood do not appear to be involved in nutritive purposes but remain as permanent constituents of the plasma.

ANTIBODIES

A foreign protein introduced into an animal is an antigen.   It elicits the formation of antibodies.   The antibodies fit precisely the surface configuration of the antigen (1).   If later these same antigens invade the animal, the specific antibodies already present can combine with the antigens and render them harmless (2) (see Fig. 3.1).   The main components of blood plasma and their functions are summarized in Table 3.1.
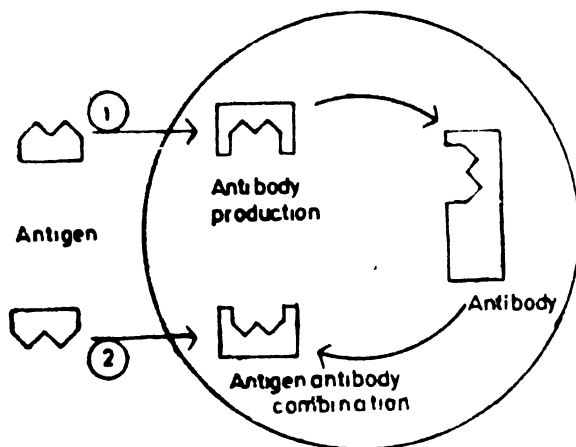
Fig. 3.1

TABLE 3.1 THE CHIEF COMPONENTS OF BLOOD PLASMA AND THEIR FUNCTIONS

Components	Functions
1. Water	Maintains blood volume and pressure; forms lymph; water supply of cells; provides vehicle for other constituents
2. Mineral ions	Maintain osmotic balance, pH balance; buffer capacity; varied effects on tissue cells
3. Plasma proteins Fibrinogen Albumins, enzymes Globulins	All maintain osmotic and pH balance Participates in blood clotting Functions obscure Basis of blood types; act as antibodies
4. Glucose, other organic metabolites	In transit to and from cells
5. Urea, CO_2, O_2, various food, hormones, vitamins, and others	In transit to and from cells

Categories 1 to 4 are maintained at constant concentrations and materials in category 5 occur in variable concentrations.

Blood Cells

WHITE BLOOD CELLS

Nonpigmented cells of various kinds and pigmented cells are present in the bloods of most animals. The nonpigmented cells are usually capable

of amoeboid locomotion. These are called white blood cells (WBC) in vertebrates. These cells can squeeze in between the cells that form the walls of the smallest blood vessels and leave the circulation in this manner. White blood cells are of two types—leukocytes and lymphocytes. The amoeboid habit is developed particularly well in leukocytes. Once the leukocytes are out of the body tissues, they are capable of migrating towards the sites of infection where they engulf the bacteria present. Pus is an accumulation of leukocytes, bacteria and cellular debris.

Lymphocytes also function in body defence. They play an important role in wound healing and scar-tissue formation after injury by transforming to messenchyme cells and fibrocytes. Lymphocytes also serve as lymph purifying agents. Particles of dust, smoke, and other materials frequently get into lungs; these are carried by lymph to the lymph nodes present along the paths of lymph vessels to be engulfed and retained there permanently.

The white blood cells do not contain any haemoglobin and differ from red corpuscles in many other important respects. They possess a nucleus and are therefore true cells. They have the power of active amoeboid movement which aids in their passage through the walls of blood vessels and enables them to travel within the connective tissues. They are much less in number than the red cells—at the proportion of one white cell to 600 red cells or about 8,000 per cubic millimeter of blood, with a normal variation from 6,000 to 10,000, a number that increases greatly under pathological conditions such as infections (leukocytosis).

More rarely is there a reduction in number (leukopenia). At birth the leukocytes are more numerous, 15,000 to 18,000 per cubic millimeter. The control system that regulates the number is not fully understood as yet. Lymph nodes are the sites where lymphocy are manufactured mainly. Leukocytes originate in the liver and spleen during embryonic stages but in the marrow of long bones in the adult. Bone marrow is also believed to be the main generating tissue of the platelets of vertebrate blood. These bodies are cell fragments, often without nuclei. Their number, too, is normally constant. A cubic millimeter of human blood contains about 250,000 platelets on the average.

The blood platelets or thrombocytes are colourless oval disks and their number normally ranges from 200,000 to 400,000 per cubic millimeter of venous blood. In arterial blood the number is about 12 per cent higher than in venous blood and the number in cutaneous blood is about 15 per cent lower than in venous. The number of platelets increases after haemorrhage and may decrease in some types of purpura, a condition associated with subcutaneous extravasation of blood. The volume of blood platelets ranges from 0.35 to 0.56 ml per 100 ml of blood. The platelets vary from 1.8 to 3.6 μ in diameter.

The chemical composition of platelets is not much known except that they contain protein and a considerable amount of phospholipid, much of which appears to be cephalin. Platelets disintegrate when blood is shed

and contribute thromboplastic substances necessary for the activation of blood protein prothrombin to thrombin in the coagulation process in the presence of calcium ions and thrombokinase. Thrombin then reacts with another of the blood proteins, fibrinogen, yielding insoluble coagulated protein, fibrin in blood clot. Vitamin K participates in some manner in the clotting process.

In pure form a clot is yellowish white mesh work of fibres, but pigmented blood cells are usually trapped in this mesh work, hence the clot is normally red. When any of the ingredients are missing or inoperative clotting cannot occur. For example, fibrinogen can be removed fairly easily from whole blood or plasma which prevents clotting—a procedure often used in storing blood or plasma for transfusions. Plasma minus fibrinogen is blood serum. Clotting is also inhibited in the absence of calcium or when blood platelets are defective. In one type of (hereditary) disease in man, platelets are not being produced. In another, platelets have thickened membranes that do not rupture on contact with obstructions. In either of these bleeder's diseases the slightest would can be fatal.

The erythrocytes spend their life-span in the blood stream, where their function is performed. They are found outside the blood vessels only under abnormal conditions or when they are about to disintegrate. The leukocytes behave quite differently. They function in the connective tissue where they are wandering elements. They arise, function and die outside of the blood stream which serves as a means of transportation from their place of origin to their destination in the connective tissues.

The leukocytes can occur in two forms—the mongranular forms, the agranulocytes and the granular form, the granulocytes. The mongranular leukocytes include the lymphocytes, which are small cells about the size of erythrocytes, and a group of larger cells, monocytes, having more cytoplasm and a more indented nucleus. The mongranular leukocytes are comparatively undifferentiated and can reproduce by mitosis. The lymphocytes constitute about 20 to 25 per cent of the white blood cells.

Monocytes are large cells constituting about 3 to 8 per cent of the leukocytes. It varlies from 12 to 15 μ in diameter. In the body, they migrate readily through the capillary walls into the connective tissues where they display their phagocytic characteristics. The monocytes function as the chief cells in the combating tubercular bacillus.

The granular leukocytes have specific types of granules in their cytoplasm and they have been subdivided according to the nature of this granulation, into three groups—the neutrophilic, eosinophilic, and basophilic leukocytes. The neutrophils vary in size from 9 to 12 μ and are the most numerous of white blood cells, constituting 50 to 75 per-cent of total white blood cells in normal individuals. The range becomes greater under ₒpathological conditions.

The eosinophilic leukocytes normally constitute from 2 to 4 per cent of the white blood cells. They are somewhat larger than the neutrophils, having a diameter 10 to 14 μ. The eosinophils increase greatly in allergic

conditions such as hay fever and asthama, in skin diseases and in parasitic infestations. The basophilic leukocytes are present in almost negligible quantity—0.5 to 1 per cent or even less of the total number of leukocytes. In size they vary from 8 to 10 μ.

The various leukocytes (WBC) are shown in Table 3.2.

TABLE 3.2 THE LEUKOCYTES

Type	Size	Cell per cubic mm of blood	
Neutrophils	10–15 μ, polymorphonuclear	3,000–7,000	(54–62%)
Eosinophils	10–15 μ, polymorphonuclear	50–500	(1–3%)
Basophils	10–15 μ, polymorphonuclear	0–50	(0–0.75%)
Monocytes	12–20 μ, single large nuclear mass	100–600	(3–7%)
Lymphocytes	10–20 μ, single large nucleus	1000–3000	(25–33%)

RED BLOOD CORPUSCLES

The red blood corpuscles or erythrocytes are highly differentiated and specialized for the function of transporting oxygen. In the lower vertebrates, the erythrocyte is a nucleated cell, but in man and all other mammals it is unique in that it loses normally its nucleus, Golgi apparatus, centrioles and most of its mitochondria during the process of maturation before entering the blood stream as a functional element. Individual red corpuscles appear greenish yellow in colour when fresh preparations of blood are examined under microscope, in masses they give a red colour characteristic of blood.

Pigmented blood cells of vertebrates are manufactured in the liver and spleen of embryos and in the bone marrow of adults. In mammals the nuclei of the cells disintegrate as they mature. The resulting red corpuscles remain in the circulation for only limited periods, in numbers that are maintained constant. The spleen and to some extent also the liver destroy corpuscles, while bone marrow manufactures them. The amount of oxygen in the environment is the rate-controlling factor. A high oxygen content in the air slows the production rate and increases the destruction rate, whereas a low oxygen content, as at high altitudes, has the opposite effect. In human blood, it has been estimated that some 10 million corpuscles are destroyed and just as many are manufactured every second. A cubic millimetre of blood normally contains more than 5 million corpuscles, thus representing the most abundant cellular blood component. The spleen of many adult vertebrates, man not included, serve to some extent as a

blood-storing organ: when it contracts it squeezes stored blood into the circulation.

The average diameter of erythrocytes is 7.7μ. They are larger in the living state (about 8.6μ) and smaller in sections (about 7μ). Large erythrocytes are commonly found in some types of anaemia (pernicious anaemia) and are known as macrocytes or megalocytes. Small forms are characteristically present in some other types or anaemia (iron deficiency anaemia) and are known as microcytes.

Chemically, the erythrocyte consists of a protein and lipoid colloidal complex, of which the most important element is haemoglobin. Pigmented blood cells derive their colour from respiratory pigments dissolved in their cytoplasms. The pigments serve primarily in oxygen transport, but carbon dioxide can be carried to some extent too. Respiratory pigments are largely of four different types: haemoglobin, haemerythrin, chlorocruorin, and haemocyanin. Haemoglobin (Hb) is by far the most widespread. It is present in all animal phyla except the sponges and the radiates. The compound consists of the iron-containing pigment haem, chemically similar to the cytochromes, and of the protein globin. When red blood cells are destroyed in the liver the iron of haem is salvaged for renewed use, and the rest of the haem molecule appears in modified form both in the faeces, via bile, and in urine, via blood and kidneys, hence the characteristic colours of these elimination products.

Haemoglobin has the remarkable property of binding oxygen in a very loose combination (oxyhaemoglobin). The haemoglobin becomes saturated with oxygen in the capillaries of the lung, and the circulating blood distributes this oxygen to the cells of the body in exchange for carbonic acid which constantly accumulates in the tissues. The corpuscle does not yield the oxygen directly to the cells, the oxygen is first dissolved in the plasma at a level held constant by the erythrocytes.

HAEMOLYSIS

The contents of the corpuscle are normally in osmotic equilibrium with the plasma and hence plasma is said to be isosmotic or isotonic. Isotonic solutions are prepared for study of the corpuscles outside the body. 0.85 per cent solution of sodium chloride is approximately isotonic for mammalian blood. When hypertonic solutions are added to blood, the erythrocytes become shrunken and crenated. This is explained by the fact that the membranous coverings of the corpuscles are permeable to water and impermeable to sodium and potassium ions and therefore water passes from the corpuscles in order to restore the osmotic equilibrium between the corpuscles and the surrounding medium whenever the latter is hypertonic.

When blood is placed in distilled water or any hypotonic solution, water enters the corpuscles and they assume a spheroidal shape. The corpuscles lose their colour by the escape of haemoglobin into the diluted

plasma, and the colourless part which remains is known as stroma, blood shadow or ghost.

Eventually, the shadows may also undergo solution. The process of extraction of haemoglobin is known as haemolysis, and the substances which effect it are known as haemolysins or haemolytic agents. Hypotonic solutions are not the only substances which produce haemolysis. The plasma of one species may haemolyze the erythrocytes of another and in man, the serum of certain individuals may produce haemolysis in others. Haemolysis is of interest in clinical work. One of the types of anaemia, haemolytic anaemia, occurs when the erythrocytes within the body are haemolysed at a rate which exceeds that of their formation.

Agglutination or clumping of corpuscles is also brought about by certain substances. During certain pathological and experimental conditions. agglutination may take place, producing a multiple thrombosis of the smaller vessels. Agglutinins present in the serum of some individuals may bring about an agglutination of erythrocytes in others. On this basis, individuals have been divided into several blood groups. It is, therefore, important to select donors from a blood group which is compatible with that of the recipient with a view to avoiding accidents which would result in giving blood transfusions, if the recipients' serum agglutinated the transfused donor cells.

Normally, haemolysis of more susceptible cells begin at salt concentrations of 0.45 to 0.39 per cent and is complete at concentrations of 0.33 to 0.30 per cent. The fragility test is based upon determination of the resistance of the cells to haemolysis as the salt concentration of the suspending fluid is lowered. Haemolysis in pure water proceeds rather rapidly. It is greatly increased by fat solvents, like ether and chloroform, which dissolve stroma lipids, and by saponin, bile salts, soaps, and other wetting agents, which tend to break up the lipid-protein complexes of stroma. Haemolysis is also promoted by acids and alkalies. The venoms of certain poisonous snakes contain powerful haemolysins. Various bacteria also form very active haemolytic agents.

RH FACTOR

The blood of an animal sensitized to the erythrocytes of another animal contains the immune bodies, haemolysins, which haemolyze the antigenic red cells. The haemolysis of erythrocytes in relation to the Rh factor is very important in medicine. The Rh substance is so called as it was first detected in the antibodies formed in guinea pigs by injection of the red cells of the rhesus monkey. The Rh substance is present in the erythrocytes of about 85 per cent of the white population. The Rh factor or principle acts as an antigen. The erythrocytes of an Rh^+ individual may contain one of several Rh antigens more or less like the Rh substance found in monkey cells. Human serum normally does not contain the antibody to the Rh factor, and the latter must be detected by the use of specially

prepared animal serum or serum from an Rh⁻ individual who has been exposed to the Rh antigen.

If an Rh⁻ person receives a blood transfusion from the Rh⁺ donor, the Rh⁻ individual develops antibodies against the Rh⁺ red cells. A subsequent transfusion of Rh⁺ blood into such a sensitized person may cause haemolysis of the Rh⁺ red cells with severe consequences. The Rh factor may be inherited. An Rh⁻ mother may have an Rh⁺ child through paternal inheritance. The Rh antigen from the foetus during such a pregnancy, may cause the production of antibodies in the mother, which passing to the foetus may cause destruction of the erythrocytes with disastrous results. Such a mother may suffer severe reactions if given a transfusion of Rh⁺ blood. All these indicate the importance of the determination of the Rh characteristics of blood.

A few of the erythrocytes of peripheral blood have a reticulated appearance when supravitally stained with cresyl blue. They are known as reticulocytes or reticulated erythrocytes. They are the youngest erythrocytes in the circulating blood and their reticulated appearance is apparently produced by a clumping of ribosomes by the supravital dye.

The erythrocytes are much more in number than any of the other formed elements. The average per cubic mm of blood in a normal adult male and a normal adult female are 5,000,000 and 4,500,000 respectively. Variations take place normally with physiological changes. It increases after exercise. Life in high altitudes is accompanied by an increase to about 8,000,000. Pronounced variations occur under pathological conditions, where not only the number but the size, shape and haemoglobin content of the corpuscles may vary strikingly. The normal number may be present, but the haemoglobin content is reduced as it happens in some of the secondary (chlorotic) anaemias. In the macrocytic anaemias—pernicious anaemia, which results from a deficiency of an erythrocyte maturation factor, Vitamin B_{12}—the red cells are reduced in number but are abnormally large (macrocytes) and some of the cells have an increased haemoglobin content. The number and the size of the cells decrease in microcytic anaemia (iron deficiency anaemia). The cells may show a multiplicity of distortions in shape (poikilocytosis) under most of these conditions.

ERYTHROCYTE SEDIMENTATION RATE (ESR)

The corpuscles settle progressively to the bottom leaving clear plasma above when blood, kept fluid by means of an anticoagulant, is allowed to stand in a narrow tube. The rate at which this takes place is very constant in health and is known as the sedimentation rate (ESR). The corpuscles settle because their density is greater than that of the plasma.

A long narrow graduated tube is filled with citrated blood (4 parts blood and 1 part sodium citrate, 3.8 per cent) to the 10 cm mark and kept at 22-27°C for an hour. The upper level of the red cells is read and the height in mm of the column of clear plasma is noted. Normally the height

of this column is 0-6.5 mm in 90 per cent of normal males and 0-12 mm in normal females, lower red cell count accounts for more rapid sedimentation rate. The average for males is 4 mm and for females 8 mm. Pregnancy raises the rate from the third month upto parturition. The sedimentation rate may exceed 50 mm in pathological conditions associated with inflammation or tissue destruction, such as acute or chronic infections of any severity or severe trauma. This increase is usually associated with an elevated fibrinogen level and occasionally a rise in globulin. Increased ESR suggests organic disease even in absence of other signs. The ESR determination is particularly of value in assessing prognosis or in judging progress or the effects of treatment. A rising ESR suggests worsening of the condition or onset of complications. The course of such diseases as tuberculous infections and rheumatoid arthritis, can be conveniently judged by the determination of sedimentation rate.

In nephrosis the sedimentation rate is increased with a considerable reduction in serum albumin. The serum globulin is generally increased when there is pronounced lipaemia as the lipids in the serum are attached to the globulin.

Haemoglobin

Haemoglobin consists of the protein globin united with the pigment haem. The haem proteins, to which haemoglobin belongs, constitute one of the most important class of biological substances. They are almost universally persent in aerobic organisms but are absent in anaerobic forms. In some form or other they occur in both plants and animals. The respiratory proteins of animals are among the haem proteins—haemoglobins, myoglobins, erythrocruorins, and chlorocruorins.

The haem proteins are formed by the conjugation of proteins with haem,

Pyrrole
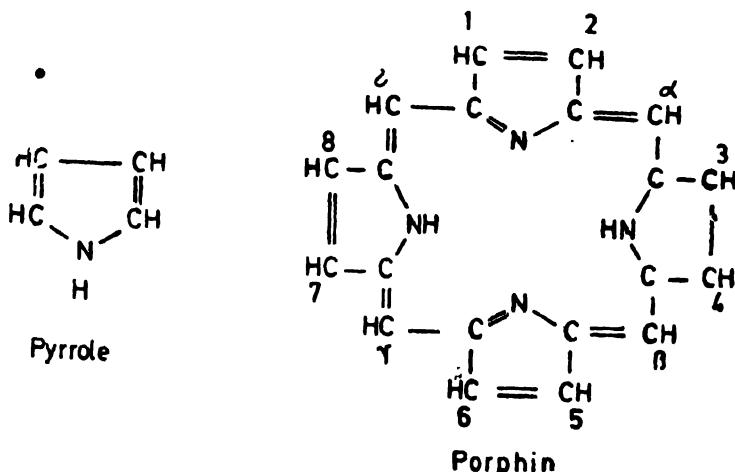
Porphin

an iron-porphyrin compound serving as the prosthetic group. The porphyrins constitute an important class of substances to which both haem and the chlorophylls belong. Porphin is the parent compound from which the porphyrins are derived. Porphin is a ring compound composed of four pyrrole groups joined together by four methine or methylidyne bridges (=CH—).

The porphins are tetrapyrroles. In haem the pyrrole rings are numbered 1, II, III, IV and the carbon atoms of the methine bridges are labelled α, β, γ and δ. The positions to which side-chains are attached are numbered (1)–(8); at (1) the side-chain is methyl (—CH₃); at (2), Vinyl (—CH=CH₂); at (3), methyl (—CH₃); at (4), Vinyl (—CH—CH₂); at (5), methyl (—CH₃); at (6), propionic acid (—CH₂—CH₂—COOH); at (7), propionic acid (—CH₂—CH₂—COOH); and at (8) the side chain is methyl (—CH₃). The side chains at (1), (3), (5), and (8) are methyl; (2) and (4) are vinyl and (6) and (7) are propionic acid. The prophyrins vary by presence of different substituent groups in the side-chains at positions (1)–(8) and the presence or absence of a metal. With iron it is haem and with magnesium, chlorophyll.

The iron in haem enters the centre of the porphyrin ring and forms bonds with the four nitrogen atoms of each pyrrole ring and to the iminazole group in the associated globin through a bond available for loose union with oxygen (in oxyhaemaglobin) or carbon monoxide (in carboxyhaemoglobin better called carbon monoxyhaemoglobin). In oxidized haemoglobin this place is taken up by an OH group on the ferric (Fe^{+++}) atom.
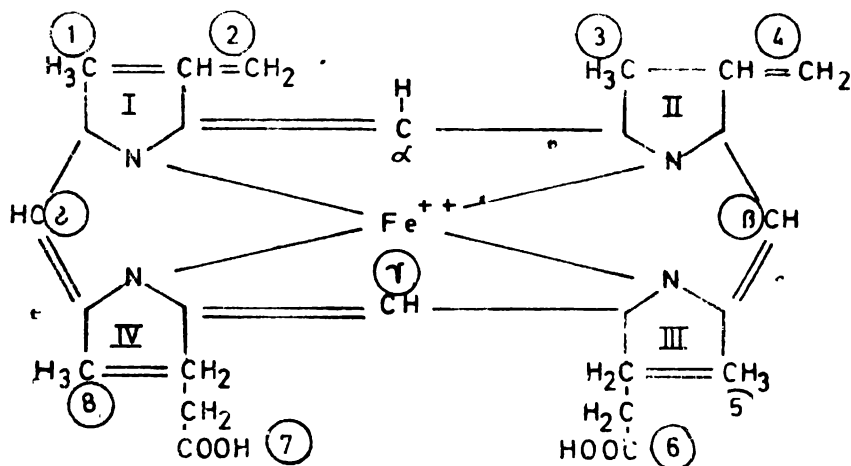
Haem - (iron protoporphyrin IX)

Fischer and associates (Fisher, H; Treibs, A; and Zeile, K.: J physiol. Chem; 193, 138, 1930) synthesized the protoporphyrin and then introduced iron into it to form haem.* Since they introduced ferric iron, the

*H. Fisher, A. Treibs and K. Zeile, *J. Physiol. Chem.*, 193 (1930), p. 138.

compound formed was ferrihaem or ferriprotoporphyrin. This was obtained as the chloride called the haemin:
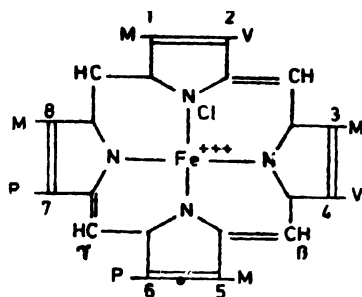
Haemin (Ferrihaem chloride, ferriprotoporphyrin)

Haemoglobins, the respiratory proteins of vertebrate erythrocytes, are formed by conjugation of basic proteins—globins—with ferrohaem (ferroprotoporphyrin). Haemoglobin is built from four polypeptide chains, of two types each in duplicate. These are intricately convoluted about an axis of symmetry to form a roughly apple-shaped molecule. Each chain is associated with one haem group (four haems to the molecule) of molecular weight 68,000. The ability with which haemoglobin combines loosely and reversibly with oxygen depends upon the ferrous (Fe^{++}) atoms of the haems—each Fe^{++} combines with one molecule of oxygen (O_2). Present knowledge of haemoglobin structure is not enough to explain the mechanism of oxygen carriage or the relative oxygen affinities of the four haem groups.

When reduced or oxygenated haemoglobin is treated with an oxidizing agent, the ferrous (Fe^{++}) is oxidized ferric (Fe^{+++}) iron, the sixth bond becomes attached to OH. The compound formed is methaemoglobin which cannot unite reversibly with gaseous oxygen and the O of the attached OH is not given off in a vacuum. Reduced haemoglobin is commonly represented as Hb; oxyhaemoglobin as HbO_2; methaemoglobin as HbOH.

By use of isotopic tracers it has been shown that duck erythrocytes synthesize haem completely from glycine and succinate, glycine providing 8 carbon atoms and nitrogen and succinate supplying 26 carbon atoms.

CH_2NH_2
|
$COOH$
Glycine

2 molecules of δ-amino-laevulic acid

Porphobilinogen

Summary of steps in the biosynthesis of porphobilinogen from Succinate + glycine.* Porphobilinogen contains single pyrrole ring and four molecules of porphobilinogen can combine to form one molecule of porphyrin.**

The haemoglobin of various species are different as revealed in their crystal structure. The differences arise from variations in the amino acids of the globin part of the molecule, the haem component remain the same in all haemoglobins. Chemical differences give rise to changes in physical properties such as solubility, affinity for oxygen, and other characteristics. Human haemoglobin contains 0.34 per cent iron having a molecular weight 68,000 with four haem groups.

The haemoglobin molecule consists of four globin peptide chains, each combined with a haem group, and all held together in definite arrangement or conformation primarily by hydrogen bonds. Salt linkages and van der Waals' forces are also involved. Concentrated urea solutions, concentrated salt solutions, solutions at pH below 6 and above 9.5, cause dissociation of the haemoglobin molecule. Acid treatment followed by column chromatography, electrophoresis and countercurrent distribution bring about the separation of the individual peptide chains involving removal of the haem groups. The haemoglobin has been found to be composed of four peptide chains, each combined with haem while myoglobin is made up of a single globin chain combined with haem.

Various normal and abnormal haemoglobins contain four kinds of globin peptide chains—α, β, γ, and δ. The amino acid sequences of human α, β, and γ chains have been determined, α-chain containing 141 amino acid residues while the β, γ, and δ chains have 146 each. The sequence of amino acids in δ chain has partially determined.

*C. Rimington, *Brit. Med. Bull.* 15 (1959), p. 19.
**E. Antonini, *Physiol, Rev.*, 45 (1965), p. 123.

The amino acid sequences of α, β, and γ chains of haemoglobin are:

10
A: α = Val.leu-Ser-pro-ala-asp-lys-thr-asg-val-lys-ala-ala-try-gly-lys-val-gly.
20
ala-his-ala.gly.glu-tyr-

10
β = Vol.his.leu.thr.pro.glu.gtu.lys.ser.ala.val.thr.ala.leu.try.
20
gly.lys.val.asg.val.asp.glu.val.gly-

10
γ = gly.his-phe-thr-glu.glu.asp.lys.ala.thr.ile.thr.ser.leu.try.gly.lys.
20
val.asg.val.glu.asp.ala.gly-

30   40
B: α = gly.ala.glu.ala.leu.glu.arg.met.phe.leu.ser.phe.pro.thr.thr.lys
thr.tyr.phe.pro.his.phe.asp.leu-
30   40
β = gly.glu.aia.leu.gly.arg.leu.leu.val.val.tyr.pro.try.thr.glm.arg.
phe.phe.glu.ser.phe.gly.asp.leu-
30   40
γ = gly.glu.thr.leu.gly.arg.leu.leu.val.val.tyr.pro.try.thr.glm.arg.
phe.phe.asp.ser.phe.gly.asg.leu-

50   60
C: α = Ser.his.gly.ser.ala.glm.val.lys.gly.his.gly.lys.lys.val.ala.asp.ala.
70
leu.thr.asg.ala.val.ala.his-
50   60
β = Ser.thr.pro.asp.ala.val.met.gly.asg.pro.lys.val.lys.ala.his.gly.
70
lys.lys.val.leu.gly.ala.phe.ser-
50   60
γ = Ser.ser.ala.ser.ala.ile.met.gly.asg.pro.lys.val.lys.ala.his.gly.lys.
70
lys.val.leu.thr.ser.leu.gly-

80
D: α = Val.asp.asp.met.pro.asg.ala.leu.ser.ala.leu.ser.asp.leu.his.ala.
90
his.lys.leu.arg.val.asp.pro.val-
80
β = asp.gly.leu.ala his.leu.asp.asg.leu.lys.gly.thr.phe.ala.thr.leu,
90
ser.glu.leu.his.cys.asp.lys.leu-

80
γ=asp.ala.ile.lys.his.leu.asp.asp.leu.lys.gly.thr.phe.ala.glm.leu.
90
ser.glu.leu.his.cys.asp.lys.leu-

100   110
E: α=asg.phe.lys.leu.leu.ser.his.cys.leu.leu.vla.thr.leu.ala.ala.his.
120
leu.pro.ala.glu.phe.thr.pro.ala-

100   110
β=his.val.asp.pro.glu.asg.phe.arg.leu.leu.gly.asg.val.leu.val.cys.
120
val.leu.ala.his.his.phe.gly.lys-

100   110
γ=his.val.asp.pro.glu.asg.phe.lys.leu.leu.gly.asg.val.leu.val.
120
thr.val.leu.ala.ile.his.phe.gly.lys-

130
F: α=val.his.ala.ser.leu.asp.lys.phe.leu.ala.ser.val.ser.thr.val.leu.
140 141
thr.ser.lys.tyr-arg-

130
β=glu.phe.thr.pro.pro.val.glm.ala.ala.tyr.glm.lys.val.val.ala.
140   146
gly.val.ala.asg.ala.leu.ala.his.lys.tyr.his-

130
γ=glu.phe.thr.pro.glu.val.glm.ala.ser.tyr.glm.lys.met.val.thr.gly.
140   146 •
val.ala.ser.ala.leu.ser.ser.arg.tys.his.

The sequence of the δ chain appears to be identical with that of the β chain except as below:

Residue No: 9   12   22   50   86   87   116   117   124 or 125   126
Amino acid: Thr-Asg-Ala-Ser-Ser-Glm-Arg-Asg — Glm — Met
                                     (?)   (?)

The organization of the haemoglobin molecule (and many other proteins) involve four kinds of structure. The amino acid sequences of the various chains represent the primary structure. The secondary structure determines certain portions of the chains formed into helices as compared to other portions that are not helical. This is determined by the amino acid sequence in each chain. The tertiary structure is represented by the folding of each chain and the relations of the various portions of the chain to other portions. The quaternary structure is represented by the

organization and conformation of all four chains in the complete haemoglobin molecule.

The haem iron atom is bound by coordination to the imidazole nitrogen of the proximal histidine residue at number 92 in the β and γ chains and at number 87 in the α chain. The haem iron is farther removed from the distal histidine residue at position 63 in the β and γ chains and 58 in the α chain, and is more loosely bound. or not bound at all, to the imidazole nitrogen. It is likely that a molecule of water may be held between the iron atom and this histidine residue and that the combination of the iron with oxygen occurs at this site. The oxygen combining power of the haemoglobin is destroyed by changes in the amino acid sequence in this region in certain haemoglobin variants.

Perutz and associates have studied intensively the organization of haemoglobin molecules from the peptide chains and haem by X-ray analysis.* The overall shape of the haemoglobin molecule resembles a spheroid having a length of 64 A°, a width of 55 A°, and a height of 50 A°. The haem groups are in four separate pockets on the molecule surface. The iron atoms in the neighbouring pockets between the α and β chains are 25 A apart, while the iron atoms of the two α chains are 36 A° and of the two β chains 33.4 A° apart. The haem groups that combine with oxygen are thus far apart.

The chloride of haem is called haemin. It is obtained when a solution of blood is heated with acetic acid and sodium chloride in the form of characteristic crystals, which serves as very good test for blood and the operation can be carried out on a microscope slide (for subsequent microscopic examination of the characteristic crystal structure of haemin for detection of blood).

Haem forms compounds with many substances other than globin, such as, haem combining with pyridine to give pyridine haemochromogen. It also combines with two molecules of other nitrogenous bases—histidine, piperidine, and nicotine to form respective haemochromogens. Purified native globin may be prepared from haemoglobin and then reacted with haem in solution to yield fully reconstituted haemoglobin.

Human haemoglobin contains 36 histidine residues with pK values near pH 7 which makes haemoglobin an excellent buffer in the physiological pH range. There are 6 SH groups in human haemoglobin one in each α and 2 in each β chain. Only two of these however, readily react with —SH group reagents. The haemoglobin molecule contains about 36 negatively and 36 positively charged groups, which are not titrable in the native molecule but become titrable when haemoglobin is denatured by acid, which makes the chain dissociated and uncoiled.
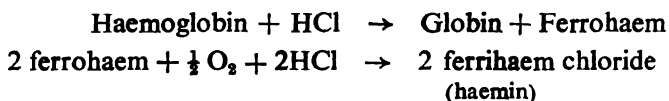
Haemoglobin possesses one of the most unique properties of any compound found in nature: its ability to combine reversibly with molecular

*M.F. Perutz, *Proteins and Nucleic Acids: Structure and Function* (New York: Elseirer, 1962).
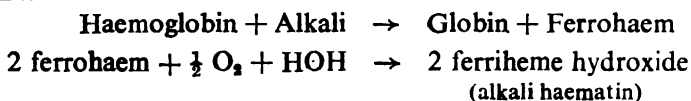
oxygen. Haemocyanin, the combined protein (like haemoglobin) found in arthropods and crustaceans, can also transport oxygen, but it contains copper instead of iron and its prosthetic group is still unknown. Approximately 1 gm of haemoglobin will combine in solution with 1.36 ml of oxygen at saturated conditions. Chemical changes in the haem or in the protein portion lead to a loss of this property.

CHEMICAL PROPERTIES OF HAEMOGLOBINS

Haem is separated from globin by the action of acids and alkalies on haemoglobin. The ferrohaem is readily oxidized to ferrihaem in presence of oxygen. Dilute HCl splits haemoglobin into globin and ferrohaem, the latter is quickly oxidized to ferrihaem which holds a chlorine ion and is called acid haematin or haemin:

$$\text{Haemoglobin} + \text{HCl} \rightarrow \text{Globin} + \text{Ferrohaem}$$
$$2 \text{ ferrohaem} + \tfrac{1}{2} O_2 + 2\text{HCl} \rightarrow 2 \text{ ferrihaem chloride}$$
$$\text{(haemin)}$$

with alkalies haemoglobin is split into globin and ferrohaem, the latter gets oxidized to ferrihaem which is combined with hydroxyl ion to give alkali haematin:

$$\text{Haemoglobin} + \text{Alkali} \rightarrow \text{Globin} + \text{Ferrohaem}$$
$$2 \text{ ferrohaem} + \tfrac{1}{2} O_2 + \text{HOH} \rightarrow 2 \text{ ferriheme hydroxide}$$
$$\text{(alkali haematin)}$$

OXYHAEMOGLOBIN HbO_2

The haemoglobin in blood is primarily concerned with the transport of oxygen from the lungs, where the oxygen pressure is high, to the tissues for utilization where the oxygen pressure is low. This is accomplished through the formation of a dissociable haemoglobin-oxygen complex—oxyhaemoglobin (HbO_2):

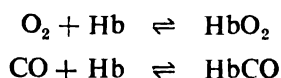$$\text{Haemoglobin} + O_2 \rightleftharpoons \text{Oxyhaemoglobin}$$
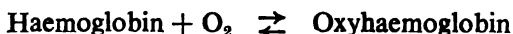
The reaction shifts to the right by an increase in oxygen pressure (lungs) and to the left by a decrease in oxygen pressure (tissues). 95 to 96 per cent of haemoglobin in blood is converted to oxyhaemoglobin at an oxygen tension of 100 mm (pressure in lung alveoli) and the haemoglobin is said to be 95 to 96 per cent saturated with oxygen. The degree of saturation varies with the oxygen tension. For a given oxygen tension the per cent saturation of haemoglobin with oxygen decreases with increase in the CO_2 tension and vice versa. At the lungs oxygenation of haemoglobin to oxyhaemoglobin increases the acidic dissociation, so that one equivalent of oxyhaemoglobin yields 0.7 equivalent of H^+ ions for combining with HCO_3^- ions to form H_2CO_3 and liberate CO_2 which is exhaled:

$$H \; HbO_2 \;\rightleftharpoons\; HbO_2^- + H^+$$
$$H^+ + HCO_3^- \;\rightleftharpoons\; H_2CO_3 \;\rightleftharpoons\; H_2O + CO_2$$
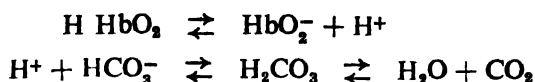
much of the CO_2 is transported from tissues to lungs by this process.

CARBON MONOXIDE HAEMOGLOBIN OR CARBOXYHAEMOGLOBIN (HbCO)

The poisonous action of carbon monoxide is attributed chiefly to its combination with haemoglobin to form carboxyhaemoglobin and thereby interfering with the oxygenation function of the haemoglobin. This is formed when the animal is exposed to CO gas. The complex is formed which is 200 times stronger than that formed with O_2. The overall reactions of O_2 and CO with haemoglobin (Hb) are:

$$O_2 + Hb \;\rightleftharpoons\; HbO_2$$
$$CO + Hb \;\rightleftharpoons\; HbCO$$

Carbon monoxide poisoning (by breathing air containing CO) can be relieved by giving pure oxygen when the conversion of carboxyhaemoglobin to oxyhaemoglobin will be promoted. The high oxygen concentration in the blood in such cases, tends to displace CO form carboxyhaemoglobin by mass action, and the CO is irreversibly exhaled by the lungs:

$$HbCO + O_2 \;(\text{high concentration}) \;\rightleftharpoons\; HbO_2 + CO \;(\text{exhaled by lungs})$$
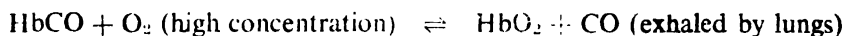
Carboxyhaemoglobin is strikingly distinguished from haemoglobin and oxyhaemoglobin by its cherry-red colour. The   in and tissues in CO poisoning are tinged with this colour. Absorption band examination serves for the identification of different haemoglobin complexes.

METHAEMOGLOBIN OR FERRIHAEMOGLOBIN (MetHb)

MetHb is obtained by the oxidation of oxyhaemoglobin or reduced haemoglobin using ferricyanide as a reagent. The compound has lost its ability to combine with molecular oxygen. It may exist in the blood to some extent due to the presence of some oxidizing agents in the circulation. There is also a hereditary disease which is characterized by the presence of MetHb in the blood.

Methaemoglobin is dark brown in colour, in contrast to purple of haemoglobin, the dark red of oxyhaemoglobin and the cherry-red of carboxyhaemoglobin. Strong reducing agents such as hydrosulphide, bring about the reduction of MetHb back to Hb.

CYANMETHAEMOGLOBIN (MetHbCN)

MetHbCN is formed by the addition of cyanide to methaemoglobin.

The compound is used for the quantitative estimation of methaemoglobin. The cyanide does not combine with oxyhaemoglobin or reduced haemoglobin. Death from cyanide poisoning is not due to loss of oxygen-carrying capacity of the blood.

Absorption of aromatic nitro or amino compounds such as, nitrobenzene, nitrophenols and aniline through skin or lungs and intake of large amounts of drugs like acetanilide and sulphonamides, may give rise to methaemoglobinemia. Cyanosis (blue skin and membranes) and dyspnea (laboured breathing) are the chief symptoms observed in severe methaemoglobinemia. The dark brown colour of methaemoglobin is responsible for the cyanosis and the dyspnea is due to the diminution in the oxygen transported to the tissues by haemoglobin.

Methaemoglobin also forms complexes with nitric oxide, azide, hydrogen sulphide, hydrogen peroxide, cyanate, thiocyanate, and fluoride. The relationships of haemoglobin and many of its derivatives are indicated in Fig. 3.2.

NORMAL BLOOD HAEMOGLOBIN

Two well-established haemoglobins are present in the blood of the normal human adult along with traces of others. The major haemoglobin is referred to as haemoglobin A which makes up 90–95 per cent of total haemoglobin. Another is haemoglobin A_2 which accounts for 2–3 per cent of total haemoglobin.

The foetal haemoglobin, haemoglobin F, is present in the umbilical cord blood of newborn infant. About 15 per cent of haemoglobin A is also present in infant's blood at birth. Haemoglobin F is exceedingly resistant to alkali denaturation which is in contrast to haemoglobin A. At birth HbF preponderates, disappearing after 2 to 3 months. In males the average haemoglobin content of blood is 15.8 g per 100 ml, in females 13.7 g. The average, irrespective of sex, is 14.5 g. In 90 per cent of normal males the range is 14–18 g and in females 12–15 g.

At birth the haemoglobin content is 23 g per 100 ml, it falls well below adult normal by the end of the third month. Recovery then gradually takes place and the haemoglobin level reaches about 12.5 g per 100 ml by the end of the first year. One gram of Hb when fully saturated combines with 1.34 ml oxygen, haemoglobin concentration is therefore an index of the oxygen-carrying power of the blood. Sometimes the haemoglobin content is referred to by an arbitrary standard called 100 per cent haemoglobin. This corresponds to 14.8 g per 100 ml and thus 50 per cent haemoglobin will be 7.4 g per 100 ml. The iron content of haemoglobin is 0.33 to 0.34 per cent.

THE ANAEMIAS

The amount of haemoglobin per unit volume of blood decreases in anaemia.

Haem
(Ferrohaem)
+
Globin

Oxdn →
← redn

Haematin
(Ferrihaem)
+
Globin

+Albumin →
← −Albumin

Methaemalbumin

Methaemoglobin
(Ferrihaemoglobin)

alkali → Alkalihaematin + Globin

Acid → Acid haematin + Globin

+CN ↓ →CN

Cyanmethaemoglobin
(Cyanferrihaemoglobin)

Haemoglobin
(Ferrohaemoglobin)

Oxdn / Redn

Alkali
+O₂ → Alkali haematin + Globin
(alkali ferrihaem)

acid
+O₂ →

Acid haematin
(acid ferrihaem)

+O₂ / +CO / −CO

Oxyhaemoglobin
(Oxyferrohaemoglobin)
+
H₂S
↓

Sulphaemoglobin + CO ⇌ Carboxy sulphaemoglobin
(Sulphferrohaemoglobin)

Oxdn → ← Redn

Sulphmethaemoglobin

Carboxyhaemoglobin
(Carboxyferrohaemoglobin)

Verdohaemoglobin

Feandglobin
removed
↓

Biliverdin
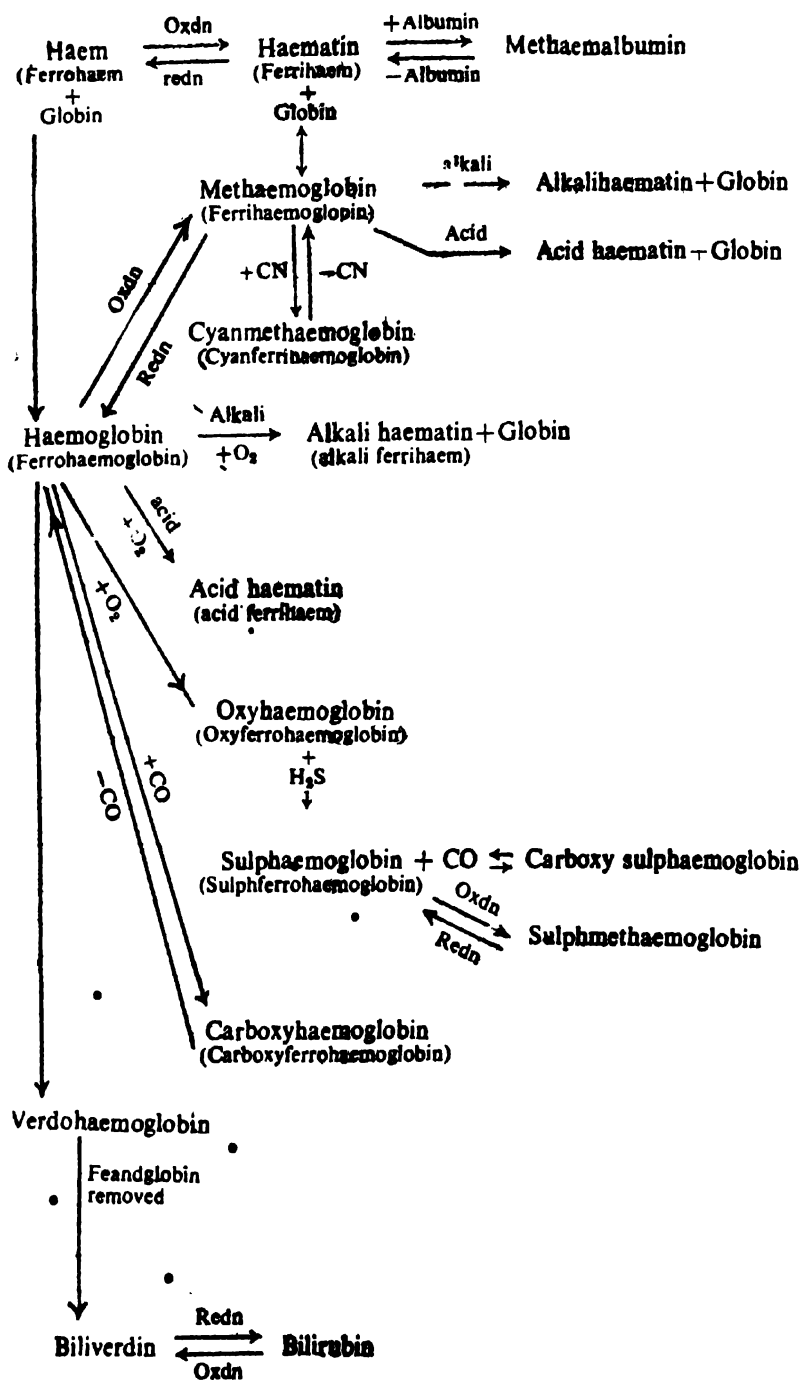
Redn →
← Oxdn

Bilirubin

Fig. 3.2 Haemoglobin and its relationship to its derivatives.

This results in a reduction in the oxygen-carrying power of the blood. The haemoglobin content in blood remains fairly constant under normal conditions despite the decomposition of the pigment and therefore the pigment formation must roughly equal pigment destruction.

The pigment production depends upon the activity of the marrow in supplying red blood cells. The elimination is brought about by the reticulo-endothelial cells—particularly in the spleen—which removes and decomposes the erythrocytes. Failure in this balance of forces is likely to result in the development of anaemia. The haemoglobin content within the normal range may decrease to 85 per cent for men and 77 per cent for women (100 per cent is referred to as $14_0 8$ g per 100 ml). The symptoms of anaemia usually appear when the haemoglobin drops from 70 per cent downwards. The red blood cell counts below 4.7 million per cubic mm (males) and 4.13 million (females) indicate abnormality.

ABNORMAL HAEMOGLOBINS

Recent experiments have brought to light the existence of a large number of human haemoglobin (Hb) variants which differ from one another in their primary structure. These differences may be the substitution of one amino acid for another in the same chain as in the helical haemoglobin A or the substitution of one chain as a whole by another as between HbA, HbF, HbH, and Hb-Bart's. Such primary structural changes may bring about secondary and tertiary changes in the coiling of the polypeptide chains and in the folding of these coils. The differences in the primary structure may result in the ultimate changes in the physical characters—the surface charge, surface structure, size, shape, intermolecular association. etc. The normal foetal haemoglobin of man, HbF, is present in infants, but it also occurs in children and adults with thalassaemia major and other abnormal haemoglobin diseases. Haemoglobin E is found either in the homozygous or in the heterozygous state in a significant proportion of the population in South-East Asia. In West Bengal, approximately 4 per cent of the people have this abnormal haemoglobin. The human haemoglobins A and F differ in solubility, in the spread on monomolecular films, in affinity for oxygen, stability towards alkalies, in ultraviolet absorption, in crystallographic behaviour, etc., electrophoretic separation of HbA and HbF, however, is not obtained*. HbE is completely separable from HbA, by electrophoresis although it does not seem to differ significantly from HBA with respect to other physical properties.**

At birth HbF preponderates, disappearing after 2-3 months. In some anaemias. however, HbF persists indicating the anaemia to be congenital. Mediterranean anaemia (thalassaemia) is an example. It is found in the homozygous offspring of parents each of whom carries the relevant gene

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*J.C. White and G.H. Beaven, *Brit. Med. Bull.*, 15 (1959), p. 330.
**G.H. Beaven and W.B. Graetzer, *J. Clin. Path.*, 12 (1959), p. 101.

(heterozygotes). ·The heterozygous carriers suffer slightly or not at all, and may have 0–10 per cent of HbF in their red cells. Severe anaemia develops in homozygous offspring and his cells may have 50 per cent HbF or above with relatively little HbA. The red cells produced are defective in other respects, and are rapidly haemolysed in the circulation. Hence the anaemia.

An electrophoretically abnormal haemoglobin was discovered by Pauling and associates in the erythrocytes of persons with sickle cell anaemia. In sickle cell anaemia the erythrocytes undergo reversible changes in shape as a result of changes of the partial pressure of oxygen. The lowering of the oxygen pressure brings about changes in the cells ,from the normal biconcave disk to crescent holly wreath or similar shapes. The process is known as sickling and this type of anaemia is hereditary.*

Many more haemoglobins have since been identified in a variety of anaemias using such technique as electrophoresis combined with chromatography. The normal human haemoglobins are A (adult) and F (foetal) respectively. Others are S (Sickle cell: HbS), C, D, H, etc. These are all under genetic control. HbF is normally present in foetal blood as well as in humans with chronic anaemia. Pauling has described the occurrence of abnormal haemoglobin as evidence of a 'molecular disease'.

FUNCTIONS OF HAEMOGLOBIN

Haemoglobin is essential for oxygen carriage. It plays an important role in the transport of CO_2 and in the regulation of blood reaction. The presence of haemoglobin in the corpuscles has its advantages. The viscosity of the plasma would have increased with rise in its osmotic pressure if haemoglobin were dissolved in the plasma. This eventuality would have deranged the mechanism of water interchange between the capillaries and the space tissues. Moreover the freed haemolobin is excreted by the kidney and taken up and destroyed by the reticuloendothelial system.

Blood Plasma

Plasma is blood from which the corpuscles have been removed. It is devoid of haemoglobin but otherwise contains much of what is present in whole blood. About 7 per cent accounts for proteins out of the 9 per cent of solids present in the plasma.

Plasma Proteins

The plasma proteins are divided into three main types—fibrinogen, albumin and globulin. Fibrinogen plays a specific role in blood coagulation. The

L. Pauling et al., Science, 110 (1949), p. 543.

proteins of the blood (particularly albumin fraction) maintain the water balance between the blood and tissues. The osmotic pressure of the plasma proteins is almost negligible when compared with the electrolytes present. Nevertheless, the electrolytes, unlike the proteins, play a less important role in the distribution of water, owing to the fact that the protein is largely confined to the interior of the cell. These proteins, more particularly the albumin, because of its smaller molecular size and presence in larger quantity, are important in the distribution of water. This is evident from the fact that patients with a deficiency in serum albumin suffer from oedema. Abnormal amounts of fluid are found in intercellular spaces, which give rise to swelling. Fluids, foods and waste products rush through the capillaries, and exchanges occur between the blood and the tissues across the capillary membrane. The tissue fluids surrounding the capillary membrane contain little protein as compared to blood plasma which contains about 7 per cent of protein. This difference in the concentration of protein gives rise to an osmotic pressure and water attempts to flow from the tissue into the capillary. This osmotic pressure is the equivalent of 22 mm of mercury approximately. A counterforce, due to blood pressure, however, tends to equalize the osmotic pressure by attempting to move fluid from the capillary to the tissue.

The blood pressure at the arterial end of the capillary is about 35 mm of mercury. This pressure is greater than the osmotic pressure (22 mm); so the fluid containing food material will pass from the capillary into the tissue and thence to cells. The blood pressure at the venous end of the capillary is only 12 mm of mercury which is considerably lower than the osmotic pressure and therefore fluid containing the waste products in the tissue and from the cell, will flow back from the tissue into the venous end of the capillary. This is the situation under normal conditions. But the situation will change under conditions in which the plasma protein has been considerably reduced below the normal level. This may be due to loss of protein from the body as it happens in nephrosis, or due to decreased intake of protein. Under this condition, the osmotic pressure drops directly in proportion with the drop in protein—fluid will flow from the capillary into the tissues not only at the arterial end but at the venous end also. The result is an abnormal accumulation of fluid in the tissue, with the development of oedema, which however rarely appears unless the serum albumin fraction falls below 2 per cent. There is little, if any, correlation between globulin and oedema.

BUFFER ACTION

The isoelectric point of albumin is pH 4.8 and that of globulin pH 5.5 and blood itself is at pH 7.4, these proteins are therefore present as anions. Plasma proteins act as buffers by virtue of their ability to accept H^+, but they account for less than one-sixth of the total buffering power of the blood. Among the proteins of the blood, the haemoglobin is the

important buffering agent. Still the albumin and globulin do help to some extent.

FRACTIONATION OF PLASMA PROTEINS

E.J. Cohn has devised methods based on fractionation with low salt concentrations at low temperatures, varying the pH and modifying conditions by the addition of alcohol in order to isolate individual plasma proteins in quantity. In this process six main functional protein fractions have been obtained:

1. Fibrinogen + antihaemophilic globulin.
2. Immunoglobulins (γ-globulins), antibodies.
3. (i) Isohaemagglutinins (β and γ-globulins).
   (ii) Prothrombin, fibrinolysin, complement (α, β and γ-globulins).
4. Angiotensinogen, alkaline phosphatase and some lipoproteins.
5. Albumin.
6. The mother liquor: albumin and β-globulin; follicle stimulating hormone of the anterior pituitary.

Many of these proteins have been isolated in a high degree of purity. Plasma albumin consists of at least two proteins and plasma globulin has been subdivided into α_1, α_2, β and γ fractions. The total plasma protein concentration ranges between 6.4 to 8.3 g per 100 ml. The average normal concentrations of the main plasma proteins in g per 100 ml are: Albumin=4.8; Globulin=2.3; Fibrinogen=0.3.

Different proteins are precipitated from solution b_ addition of different concentrations of salts. Albumin is precipitated by sa ration with $(NH_4)_2$ SO_4, globulin by half saturation with $(NH_4)_2SO_4$. Fibrinogen precipitates at 0.25 saturation, γ-globulin at 0.34 saturation, α and β-globulin at 0.5 saturation.

The plasma proteins can be separated by precipitation with various concentrations of alcohol at temperatures below 0°C. The plasma proteins are not denatured at such low temperatures and it is possible to obtain plasma proteins in a relatively pure state by this process. Electrophoresis on paper or cellulose acetate offers a reliable method for the separation and isolation of different protein fractions.

The molecular weight of plasma albumin is 69,000 and that of fibrinogen about 330,000. Not only the size but also the shape of the protein molecules determine the penetration through the capillary wall. Albumin passes more readily than the others, but all plasma proteins pass through in small amounts and appear in the lymph. All the proteins escape much more readily than normal when capillary permeability is increased (in anoxia, urticaria, inflammation).

ORIGIN OF PLASMA PROTEINS

Liver is the sole source of fibrinogen formation. The blood fibrinogen falls rapidly when the liver is poisoned with agents like chloroform and phosphorus (hepatic poisons). It return to normal on regeneration and repair of the liver. Hepatectomy similarly brings about a rapid fall in fibrinogen.

Liver is also the site of formation of most of the blood albumin and globulin. The low plasma protein levels (hypoproteinemia) associated with cirrhosis of liver provide clinical evidence of the importance of the organ in plasma protein formation, most of which being albumin and globulin. The half-life of albumin in the plasma is about 18 days and some 10 to 12 g are produced each day. Serum albumins have the important property of binding other substances such as lipids, hormones, bilirubin and many drugs.

The globulins include α_1-, α_2-, β- and γ-globulins having a wide range of molecular weights from 90,000 to 13,00,000 or more and can be separated readily by electrophoresis. They are produced mainly in the liver but γ globulins are formed in the reticuloendothelial tissue and in plasma cells and lymphocytes. α_1-globulins contain a fraction which combines with bilirubin and another fraction, the α_1-lipoprotein of the plasma, responsible in part for the carriage of lipids and steroids. The mucoproteins and the hepatoglobins are contained in the α_2-globulin fraction.

β-lipoprotein, among the β-globulins, is a very high molecular weight protein responsible for the transport of lipids in the blood. Prothrombin is a γ-globulin. All known antibodies are included in the γ-globulin fraction. Antibodies are produced mainly in the bone marrow (from the plasma cells), and in the spleen, liver and lymph nodes. Solutions of γ-globulin are frequently used so confer passive immunity. Very little γ-globulin is produced in new-born child because of the fact that the child possesses few plasma cells in lymph nodes or bone marrow. But the γ-globulin transferred across placenta from the mother protects the child. The child however, is able to produce adequate amounts of γ-globulin by the time he is nine months old.

Plasma proteins are normally formed from food proteins but in protein starvation they may be formed from tissue protein. The efficacy of a food protein depends on the degree of its chemical resemblance in amino acid pattern to that of the plasma protein which it is going to form. Plasma proteins can be synthesized if the essential aminoacids— leucine, isoleucine, methionine, phenylalanine, histidine, arginine, lysine, tryptophan, valine and threonine, are present. Tissue protein reserves are used for the formation of plasma proteins, and conversely the plasma proteins serve in the formation and maintenance of tissue proteins. A dynamic state thus exists between tissue and plasma proteins so that each may be used for the conversion into the other as occasion demands.

PLASMA LIPOPROTEINS

The lipoproteins of blood plasma have recently attracted much attention. This is largely due to the development of the lipoprotein phenotyping system of Fredrickson and his colleagues.   Their work pertains to the disorders of lipid metabolism which has proved to be of great value in the study of diseases of the heart and vascular system.   Ultracentrifugation offers a ready means for the separation of plasma lipoproteins.   Four main classes of lipoproteins have been identified by paper electrophoresis.   They are:

1.   α-Lipoprotein which consists of about 50 per cent protein, 30 per cent phospholipid and 20 per cent cholesterol.
2.   β-lipoproteins contain about 25 per cent protein, 20 per cent phospholipid, 10 per cent triglycerides and 45 per cent cholesterol and its esters.
3.   Pre-β-liproprotein which functions mainly as a carrier of triglyceride of endogenous origin which may account for 50 to 80 per cent of its composition.   Some cholesterol is present.
4.   Chylomicrons are large particles.   It is composed of dietary fat together with a small quantity of cholesterol.   They are important in relation to fat absorption from the gut.

The lipoprotein fractions may be increased in various disorders of lipid metabolism—a condition known as hyperlipoproteinemia.

ENZYMES IN BLOOD

Blood plasma contains a number of enzymes such as amylase, lipase, catalase, arginase, aldolase, adenosine polyphosphatases, β-glucuronidase, histaminase, peptidases, glutamic-aspartic transaminase, lactic dehydrogenase, and acid and alkaline phosphatase.   They originate apparently from the disintegration of tissue and blood cells.   Useful clinical information is often provided by the estimation of blood levels of some of these enzymes.

Serum amylase originates in the pancreas and salivary glands. , Acute pancreatitis, obstruction of the pancreatic duct, inflammation of the parotid gland (mumps) are characterized by markedly elevated , serum amylase level.   So is the case with serum lipase which also originates in the pancreas.

• Osteoblasts of bone are mainly responsible for the formation of a serum alkaline phosphatase (optimum pH is about 9).   It is high in Paget's disease and osteomalacia.   Certain types of hepatic diseases and obstructive jaundice also give rise to the increased serum alkaline phosphatase.   Liver may be the site for its formation in such cases.

Liver and spleen may be the sites of the formation of serum acid phosphatase (optimum pH is 4.9).   Acid phosphatase activity is present in several tissues, but adult prostate gland forms and contains the highest

concentration. The enzyme increases markedly in prostatic carcinoma.

The serum peptidases increase in conditions causing excessive tissue breakdown, such as shock, fever, traumatic injury and in haemolytic anaemia. Serum glutamic-aspartic transaminase increases markedly in liver cell damage (hepatitis) to a lesser degree and transient in myocardial infarction (coronary thrombosis). Both liver and heart cells are rich in transaminases.

FUNCTIONS OF PLASMA PROTEINS

The main functions of plasma proteins are:

1. They contribute the viscosity of plasma providing resistance to blood flow in the vascular system. This is essential for efficient heart action.
2. They serve as a source of nutrition for the tissues of the body.
3. They aid in the regulation and distribution of fluid between the blood and tissues through their osmotic effect.
4. They control haemorrhage through the mechanism of blood coagulation.
5. They contribute to the solution and transport of lipids, fat-soluble vitamins, bile-salts, hormones, etc., through the formation of complexes.
6. They provide antibodies for defence against infection (γ-globin fraction)

PROTEIN DEFICIENCY

Plasma protein in amounts below normal (5.5 g per 100 ml or below— hypoproteinemia) is also an indication of loss of body protein. The hypoproteinemia may occur due to insufficient protein intake, poor utilization, excessive loss of blood and therefore of plasma proteins. In nephritis there is a marked loss of blood albumin, giving rise to albuminurea. The hypoproteinemia is often associated with oedema and anaemia. When the blood volume is sufficiently reduced shock develops.

Total plasma proteins increase in dehydration (haemoconcentration) and in various diseases such as lymphogranuloma, venerum, granuloma inguinale, sarcoid, leprosy, and multiple myeloma. The latter disease, caused by a tumour of the bone marrow is characterized by the presence of so-called Bence-Jones protein in the plasma which may appear in the urine.

γ-globulin formation is impaired (agammaglobinemia) in a rare congenital condition. This results in lack of antibodies in persons who are particularly susceptible to infections. An interesting γ-globulin of high molecular weight, properdin, has been found to be a normal serum constituent which has the property of lysine many grams negative bacteria.

Plasma proteins, albumin, globulin and fibrinogen, may undergo alterations in many diseases with little or no abnormality in total plasma proteins content. A decrease in albumin is accompanied by an increase in globulin. Infections often increase γ-globulin due to antibody formation. Acute infections, nephrosis, cirrhosis, and and pregnancy, and X-radiation cause elevation of plasma fibrimogen. It falls sharply in some liver diseases.

The hypoproteinemia is often accompanied by oedema and anaemia. The oedema results from an increased intestinal fluid volume, because with less protein within the blood vessels, less liquid is drawn into them than would normally be the case. Shock develops when the blood volume is sufficiently reduced.

Heparin

Howell and Holt obtained an anticoagulant factor from liver in 1918 which was called heparin. Heparin is a polymer of D-glucuronic acid and D-glucosamine in which both the amino and some of the hydroxyl groups are combined with sulphuric acid which makes it a strong acidic substance. Heparin is an acidic mucopolysaccharide with a molecular weight of about 17,000.

Heparin is formed in the metachromatic granules of the mast cells of Ehrlich, which are chiefly found along blood vessel walls. It has been obtained as a crystalline barium salt from both liver and lung. Heparin acts as an anticoagulent at various stages of the coagulation process. Activation of both the antihaemophilic factor (factor VIII) and christmas factor (factor IX) and also the action of thrombin upon fibrionogen are inhibited by heparin. Heparin also inhibits the conversion of prothrombin to thrombin. It may also block the formation of thrombin from prothrombin by tissue thromboplastin.

Normal mammalian plasma does not appear to contain free heparin, it may however occur in combined and probably inactive form. Lipoprotein lipase (clearing factor) is liberated into the blood by the injection of heparin into an animal. Lipoprotein in lipase brings about the digestion of triglycerides associated with chylomicrons and lipoproteins, the turbidity of lipemic plasma being cleared up thereby. Commercial heparin is used chiefly as an anticoagulant in blood analysis and administration to patients to prevent thrombosis. /

Cerebrospinal Fluid

The cerebrospinal fluid (CSF) is formed by the choroid plexus and appears first in the lateral ventricles. It then passes to the third and fourth ventricles and finally to the subarachnoid space. 100 to 150 ml of CSF are present in the ventriculo-subarachnoid space of adults as determined by the amount that can be removed by lumbar puncture. The quantity in

the new-born varies from a few drops to 5 ml, which with age increases to adult value. The CSF is renewed several times per day.

CSF differs from plasma in composition. It is a clear colourless fluid having a low viscosity unlike lymph and plasma. It does not coagulate. The protein content is exceedingly low ranging from 15 to 55 mg per 100 ml. About 80 per cent of the protein is albumin, the remaining 20 per cent being globulin. Fibrinogen is absent. The glucose content of CSF is less than that in plasma. With a plasma glucose content at 75 to 100 mg per 100 ml, the cerebrospinal level is 47 to 78. With blood glucose value 150 to 200, the CSF will have 73 to 112 mg per 100 ml.

The calcium content in CSF is about half that found in serum (4.1 to 5.9 mg per 100 ml). Na^+ is considerably higher in CSF and K^+ a little lower than in serum. The chlorides of cerebrospinal fluid are considerably higher than in plasma and phosphate is much lower. Plasma and CSF have the same bicarbonate content and is equivalent to 40 to 60 volumes per cent CO_2. Plasma and CSF have the same pH. Cerebro spinal fluid contains 0 to 5 cells per cubic mm and in many cases the cells are not present at all.

The chemical determinations most frequently of value in the examination of cerebro spinal fluid are those of protein, chlorides, sugar, calcium and urea. Lange's colloidal gold reaction is also of importance. Globulin tests in normal fluid are negative. Total protein may be increased in many pathological conditions. In meningitis the chlorides are lowered characteristically. Sugar is also lowered in meningitis. Calcium may be lowered in tetany and the urea value closely parallels the level of blood urea.

TABLE 3.3 COMPOSITION OF CSF

Constituent	Normal range (per 100 ml)	Clinical conditions in which high values (unless otherwise stated) are found
1. Sugar (glucose)	60–100 mg	Diabetes. Reduced in acute supurative meningitis.
2. Chloride as NaCl	700–750 mg	Nephritis, Decreased in meningitis, particularly tubercular meningitis.
3. Proteins (total)	20–40 mg	Meningitis. Syphilitic conditions. Froin's syndrome.
4. Globulin (Pandy Nonne Apelt test)	Reactions negative	

Lange's colloidal gold reaction depends on the fact that although normal CSF has no action on a particular colloidal gold solution, fluid from cases of syphilis, disseminated sclerosis, or meningitis may cause various degrees of precipitation of the gold at different dilutions of the CSF, which are fairly characteristic for each disease. Typical responses are:

Luetic	0134321000	(The figures serve to indicate the degrees of precipitation in tubes 1–10 in that order).
Paretic	5554321000	
Meningitic	0011232210	

Complete precipitation (clear colourless supernatant fluid) is called   5

Partial precipitation (slightly cloudy, 'ight blue supernatant fluid) is called   4

Deep blue colour is called   3

Lilac to purple colour is called   2

Lilac colour is called   1

∴ Unchanged red colour is called   0

The dilutions of CSF are 1:10; 1:20; 1:40...1:5120 in tubes 1 to 10 made with phosphate buffer pH 6.6.

A meningitic type of curve is found in all forms of coccal meningitis and tubercular meningitis. A paretic type of curve is found in general paralysis of the insane (GPI), in tabes, in disseminated sclerosis and rarely in encephalitis lethargica. When a paretic curve occurs in association with a positive Wasserman reaction (WR) and is unaffected by antiseptic treatment it is symptomatic of GPI rather than tabes: when it occurs with a negative WR it is strongly suggestive of disseminated sclerosis. A luetic type of curve occurs in all forms of cerebral syphilis. It may also be found in disseminated sclerosis and is more common in encephalitis lethargica than in paretic type.

SYNOVIAL FLUID

Synovial fluid serves to lubricate the articular surfaces of joints. It is a clear, light yellow, viscous fluid. An average of 3.4 per cent solids, of which 2.8 per cent is protein made up of about two-thirds albumin and one-third globulin, is present in the fluid from the knee joint of man.

The hyaluronic acid content in the fluid varies from 4 to 295 mg per 100 g. It contains a number of enzymes such as, hyaluronidase (spreading factor), amylase, protease, and lipase. The electrolyte and diffusible nonelectrolyte (glucose, urea, uric acid) content of synovial fluid indicates that it is essentially a dialysate of plasma to which various substances have been added by joint tissue.

AQUEOUS AND VITREOUS HUMORS

The aqueous humor maintains the necessary intraocular pressure and provides the nutrients to the lens and cornea, which have no blood supply. It is a clear, limpid fluid which fills the anterior chamber of the eye. The aqueous humor contains about 25 mg per cent protein and diffusible

substances which are similar to those of blood transudate. It originates by flow of liquid from the posterior into the anterior chamber of the eye, secretion by the ciliary body, and diffusion from blood vessels of the iris. Aqueous humor passes from the anterior chamber through the canal of Schlemm indicating thereby that the fluid is constantly being renewed. The rate of electrolyte turnover in the aqueous humor or is much slower, involving largely sodium, which is secreted into the aqueous by the ciliary body against a concentration gradient, and represents a case of active transport or a 'sodium pump' requiring metabolic energy.

Increased secretion of aqueous may raise the intra-ocular pressure so much to cause the disease called glaucoma. The elevated pressure is lowered by the administration of the drug acetazolamide (2-acetylamino-1,3,4-thiadiazole-5-sulphonamide). The drug is an inhibitor of carbonic anhydrase indicating that carbonic anhydrase may be involved in the active transport mechanism.

The posterior chamber of the eye is filled with vitreous humor, which consists essentially of a hyaluronic acid-protein gel permeated by a fluid similar to aqueous humor. Vitrein is the protein of vitreous humor.

SEMEN

The seminal fluid or semen is a suspension of spermatozoa in seminal plasma. It is a mixture of secretions from the prostate, seminal vesicles, epididymis, urethral glands, Cowper's glands, and vasa deferentia. Semen is very viscous and clots readily due to the presence of fibrinogen but the fibronolysin of prostatic fluid promptly breaks down the clot. Human semen is alkaline with a pH varying from 7.1 to 7.5.

The fructose content of semen is characteristically high. It ranges between 90 to 520 mg per 100 ml. The semen contains a high concentration of citrate with an average of about 480 mg per cent. The acid phosphatase content is enormous ranging between 54,000 to 420,000 units per 100 ml, contributed by the prostatic fluid. Amylase, cholinesterase, β-glucuronidase, hyaluronidase, 5'-nucleotidase, diamine oxidase, alkaline phosphatase, and proteases constitute other enzymes present in semen. Semen contains the basic polyamino compounds spermine, $H_2N - (CH_2)_3 - NH - (CH_2)_4 - NH - (CH_2)_3 - NH_2$, and spermidine, $H_2N.(CH_2)_3 - NH(CH_2)_4 - NH_2$ which are derived from the prostate. These compounds appear to give the characteristic odour of semen. The major proportion of seminal plasma is prostatic fluid.

EXTRACELLULAR FLUID

The extracellular fluid surrounds the intracellular of the cell proper. It consists of (1) the blood plasma and (2) the interstitial fluid. This interstitial fluid includes the lymph.
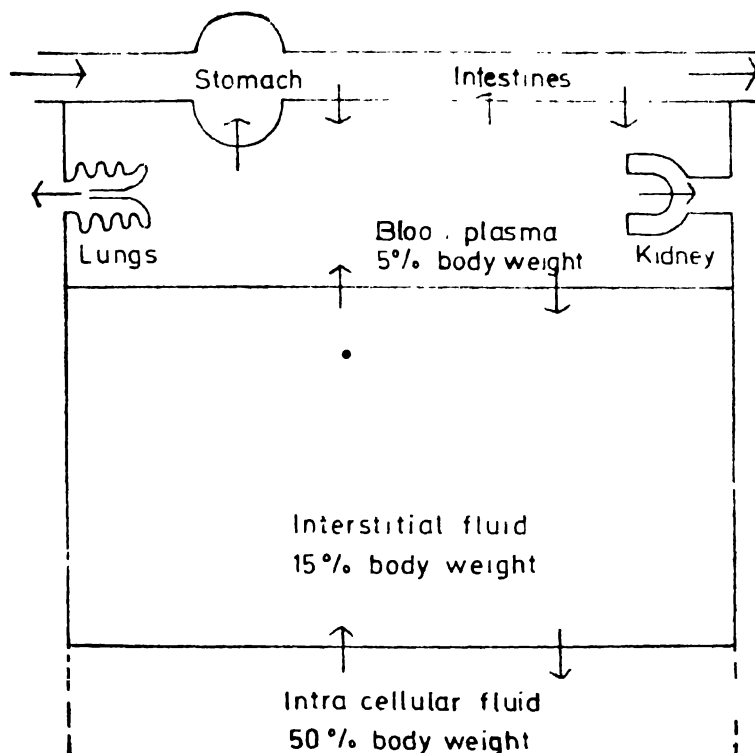
Fig. 3.3   Interstitial fluid (from Gamble,   *Extracellular Fluid*, Cambridge Mass., Harvard University Press, 1954)

The lymph, formed probably from the blood pla-ma and filling tissue spaces, acts as a medium between the blood and cells. It resembles plasma in composition. Lymph capillaries, which are in abundance in the tissue spaces, carry lymph into vessels which become larger uniting at the thoracic duct. This in turn empties into the subclavian vein, so that the products in the lymph find ultimately their way into the general circulation. One of the important functions of the lymph system is a defence against inflammatory processes.

Blood plasma and interstitial fluid have almost identical pattern with outstanding difference due to the presence of protein n the plasma. The protein of plasma is replaced in the interstitial fluid by a balanced reduction of cation and increase in diffusible anion in order to maintain osmotic equivalence between these two fluid compartments. Owing to its multivalency, the chemical equivalent of protein (in terms of osmotic effect) is about eight times its concentration value. There is a difference in the distribution of Na and K ions between the extracellular fluid and the intracellular fluid.

Inulin when injected intravenously, passes through the capillary walls and is distributed throughout both plasma and interstitial fluid, and the
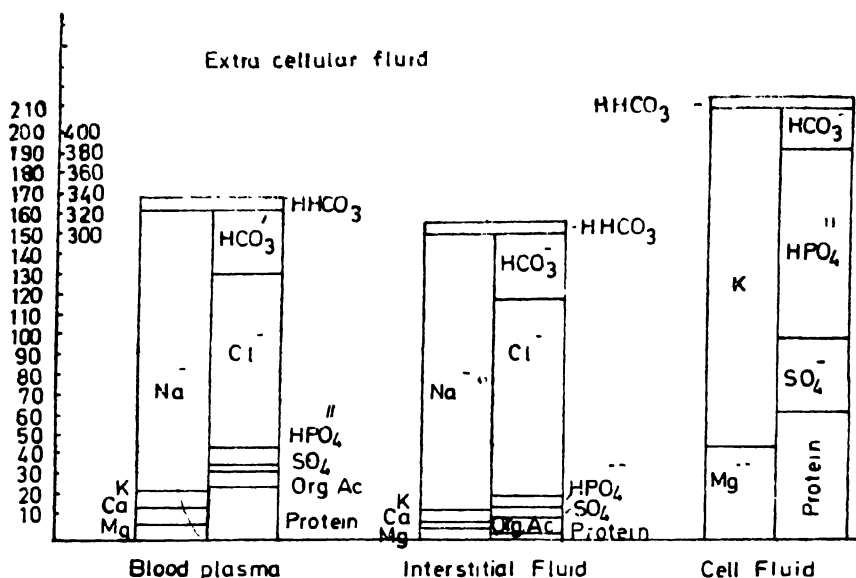
Fig. 3.4. Electrolyte composition of Blood Plasma, Interstitial Fluid and Intracellular Fluid. Values for cations (potential base) are in the left hand columns, and values for anions (acid radicals) are in the right hand columns. (From Gamble, *Extracellular Fluid* (Cambridge, Mass: Harvard University Press, 1954)

inulin space is determined by dilution of the injected sample. This represents both plasma and interstitial space. However, injected radioactive Iodine (I^{131}) albumin is retained within the vascular system and the I^{131} albumin space represents plasma space. The interstitial fluid space is thus the difference between the inulin space and the I^{131} albumin space. Plasma is about 5 per cent and the interstial fluid about 15 per cent of the body weight according to such determinations.

The parenchymal cells of organs are bound together by intercellular cement of gel consistency containing hyaluronic acid through which interstitial fluid permeates. This cement acting as a barrier prevents the passage of large molecules, such as proteins, and to bacteria, viruses, and particulate matter while small electrolyte ions, small organic molecules, and water are permitted ready passage.

Various microorganisms secrete the enzyme hyaluronidase, which break down the cement barrier by depolymerizing hyaluronic acid. This permits the spread of infection through the tissues and hyaluronidase is referred to as the 'spreading factor'.

Lymph

The lymphatic fluid is a transudate formed from the plasma by filtration through the wall of the capillary. Lymph may be considered as the 'middle man' in the transactions between blood and tissues. It is the medium by which the nutritive material and oxygen transported by the

blood for the tissues is brought into intimate contact with those tissues and thus utilized. In the further fulfilment of its function, the lymph bears from the tissues water, salts, and the products of the activity and catabolism of the tissues and passes these into the blood. Lymph thus functions as a 'go-between' for blood and tissues. It bathes every active tissue of the animal body and is believed to have its origin partly in the blood and partly in the tissues. The tc; il lymph flow into the blood through the lymph ducts appears to be 1 'ɔ 2 litres per day in an average human adult.

Lymph resembles blood plasma in chemical characteristics. In fact, it has been termed 'blood without its red corpuscles'. Since lymph represents interstitial fluid collected in the lymphatic system from many different kinds of tissues, which modify its composition to different degrees, the composition of lymph shows marked variations according to its source. Thus, the fluid from the leg contains 2–3 per cent protein, while that from the intestine contains 4–6 per cent and that from the liver 6–8 per cent. The concentrations per unit of water of readily diffusible non-electrolytes, such as urea and glucose, are about the same in plasma, interstitial fluid, and lymph, while the concentrations of diffusible ions like K^+, Na^+, Cl^-, and HCO_3^- vary according to protein content, which determines the Donnan distribution.

Lymph from the thoracic duct of a fasting animal or from a large lymphatic vessel of a well-nourished animal is of a variable colour— colourless, yellowish, or slightly reddish—and alkaline in reaction. It contains fibrinogen, prothrombin, and leukocytes, and coagulates slowly, the clot being less firm and bulky than the blood clot. Serum albumin and serum globulin are both present in lymph, the albumi ɔ predominatiɔg in a ratio of about 3 or 4:1. The principal inorganic Its are sodium salts (chloride and bicarbonate), whereas the phosphates of potassium, calcium, magnesium, and iron are present in smaller amount. The lymph transports plasma proteins synthesized in the liver to the blood stream. Raising the venous pressure within subcutaneous tissue to 30 mm raises the protein concentration of subcutaneous lymph from 0.24 per cent to 1.3 per cent, indicating increased filtration of plasma proteins into the interstitial spaces.*

Substances which stimulate the flow of lymph are termed lymphagogues. Such substances as sugar, urea, certain salts (especially sodium chloride), peptone, egg albumin, extracts of dogs' liver and intestine, crab muscles, and blood leeches are included in this class.

The lymph coming from the intestine of a fasting animal is clear, transparent fluid. This intestinal lymph is white or milky after a meal containing fat has been ingested. This is termed chyle and is essentially lymph possessing an abnormally high (5 to 15 per cent) content of emulsified fat. This chyle is absorbed by the lacteals of the intestine and transported to

*E.A. Stead and J.V. Warren, *J. Clin. Invest.*, 23 (1944), p. 283.

the lower portion of the thoracic duct. Apart from the fat content, the composition of lymph and chyle are similar.

SWEAT

The sweat glands secrete sweat to cool the body. The autonomous nervous system and the adrenal cortical steroids which affect the quantities of electrolytes present, control the sweat glands. The insensible perspiration in which no fluid is visible on the skin, generally amounts to 800 to 1200 ml per day. The volume of sweat produced per day during muscular exercise at elevated temperatures may, however, be as much as 14 litres. The water content of sweat generally varies between 99.2 and 99.7 per cent and the pH ranges from 4.7 to 7.5. Sweat contains in small amounts many nitrogenous and other organic compounds, such as lactic acid. The total non-protein nitrogen, mainly urea, varies from about 0.07 g per day to as much as 1.0 g per hour during copious sweating. There is a wide variation in the electrolytes content of sweat—Na^+:12.6—127 meq (milly equivalent); K^+:5—32 meq; Cl^-:8.5—85 meq per litre, Na^+ and Cl^- being the chief components, although considerable K^+ may also be present. The salt concentration as well as the volume of sweat are increased by muscular exercise. The loss of large volumes of sweat with accompanying electrolytes may lead to hypertonic decrease in blood and other body fluids, causing severe cramps (miners cramps). Small amounts of salt in the drinking water prevent and relieve the symptoms.

TEARS

The lachrymal glands produce tears as a clear and limpid fluid. The fluid as secreted is isotonic but as it passes over the cornea gets evaporated to become hypertonic. The fluid is istonic when the flow is copious. The tears function to lubricate the surface of the cornea, to fill in irregularities of the cornel surface in order to improve the optical properties, and also in the protection of the eye from injury. The lachrymal glands are innervated by parasympathetic fibres, which are carried in the lachrymal nerve, a branch of the ophthalmic nerve. The lachrymal nerve fibres end in the cornea and conjunctiva.

Abnormal stimuli to the cornea or conjunctiva causes excessive lachrymation which is also caused by sneezing, coughing, and psychic stimuli. Like plasma, the tears have a pH around 7.4. The protein content of tears is about 0.7g per 100 ml with an albumin: globulin ratio of about 1.5. Tears contain small amounts of mucin derived from the conjunctiva, which is responsible for the lubricating property along with the proteins present in tears. Small organic molecules and electrolytes comparable to those in blood are also present in tears. The enzyme lysozyme occurs in tears. The cells of a number of micro-organisms are lysed by the enzyme by breaking down the mucopolysa charide component of their outer layers

TABLE 3.4 APPROXIMATE COMPOSITION OF VARIOUS BODY FLUIDS

Constituent	Blood plasma	Cervical lymph dog	Thoracic lymph	Cerebro spinal fluid	Aqueous humor	Synovial fluid	Sweat	Tears
1. Protein (g per 100 ml) •	6.5-7.5	3.6	2.8-3.6	0.028	0.025	2.8	0	0.67
2. Albumin (,,)	4.7-5.7	2.4	1.6-2.4	0.015	—	1.9	—	0.39
3. Globulin (,,)	1.3-2.5	1.3	1.2	0.013	—	0.9	—	0.28
4. Fibrinogen (,, ,,)	0.2-0.4	+	+	0	—	0	—	—
5. Aminoacids	30-50	4.8	—	7.5	—	—	11-32	—
6. Total lipid	360-820	300	200-7300	Very low	—	—	0	—
7. Cholesterol	150-250	55	75 (dog)	Trace	—	—	—	—
8. Fatty acids	295-340	240	—	1.3	—	—	—	—
9. Creatinine	1.0	1.4	1.0	1.2	—	—	—	—
10. Urea	25	23	23 (dog)	10-15	—	—	12-57	0.03
11. Glucose	65-90	132	65-90 (dog)	47-78	55-110	65-90	Reducing substance 2.8-40	2.5
12. Sodium	310-350	360	310-350	345	400-415	310	29-294	334
13. Potassium	19	—	18.3	11-16	13-24.5	16	21-126	12-33
14. Calcium	9-11	9.8	7.7	4.5-5.2	14	—	1-8	—
15. Chloride	335	4?	335	420-450	410-435	328	30-300	118-138
16. Phosphorus inorganic	3.2-4.3	5.9	3.9	1.2-2.1	—	—	0-2	—
17. CO_2 Content (Vo %)	55-75	59	—	40-60	63	—	—	60
18. pH	7.35-7.45	7.4	—	7.35-7.40	—	7.29-7.45	3.8-6.5	7.3-7.7
19. Water (g per 100 ml)	94	96	Variable with lipid content	98.3-99.2	99	96-6	99.2-99.7	98.2

thereby protecting the eye from infectious agents.

Approximate composition of various body fluids is indicated in Table 3.4. Values are in mg per 100 ml unless otherwise indicated. Cervical lymph values are for dog and other values are for man except as indicated. Averages are given by single values otherwise range is indicated. Values are meant for gross comparative purposes only and have been obtained from many sources by various analytical methods.

BLOOD ANALYSIS

The importance of blood analysis as aids in clinical diagnosis has been realized for a long time. As the methods available for blood analysis were not adapted to small quantities, progress in blood chemistry remained more or less at standstill for a long time. Blood analysis has become a very important adjunct to clinical diagnosis with the introduction of micro methods particularly of colorimeter and photoelectric colorimeter. The importance of blood analysis to take but a few examples at random, is revealed in:

1. a low iron content in various anaemias
2. hyperglycemia in diabetes
3. low phosphorus and increased serum phosphatase in rickets
4. decreased plasma prothrombin in obstructive jaundice
5. increased blood NPN (urea N, creatinine, uric acid, etc.) in renal impairment, etc.

Tests for Blood

Two of the tests for detection of blood depend upon colour production due to oxidation—the guaic test and the benezidine test. In the former guaic is dissolved in glacial acetic acid, to which on addition of the blood and hydrogen peroxide, a blue colour is formed. In the benzidine test, glacial acetic acid solution of benzidine is mixed with blood and hydrogen peroxide. A blue or green colour develops. The haemin test is the best for detection of blood but it fails to distinguish human blood from other varieties.

The immunological test is used to distinguish human blood from other varieties. Rabbits are injected with human blood serum over a period of several weeks and in increasing quantities. The rabbit develops antibodies. Blood is withdrawn from the animal and its serum is mixed with human serum under examination. A turbidity, gradually changing to a flocculent precipitate, indicates the presence of human blood.

Normal values for human blood and their deviation under pathological conditions are indicated in Table 3.5*. The abbreviations used are:

*Most of these values are taken from a comprehensive table prepared by O.Bodansky, in Bodansky and Bodansky, *Biochemistry of Disease* (1952).

TABLE 3.5 NORMAL VALUES FOR HUMAN BLOOD AND DEVIATIONS

Constituent	Mean	Units	Standard deviation	Examples of abnormal value
1. Albumin (s)	5.2	g/100 ml	0.7	Low in nephrosis
2. Amino acids (P)	4.4	mg N/100 ml	0.48	High in acute atropy of liver
3. Amylase (S)	105	Somogyi Units	26	High in acute pancreatitis
4. Ascorbic acid (P)	0.75	mg/100 ml	0.40	Low in scurvy
5. Bilirubin (S)	0.54	mg/100 ml	0.25	High in biliary obstruction
6. Calcium (S)	10.0	mg/100 ml	0.36	High in hyper-, low in hypo-parathyroidism
7. CO_2 Content, Venous (S)	28.4	m Mol/litre	2.7	Low in diabetic acidosis
8. Chloride (S)	104	m Eq/litre	2.6	Low in pernicious vomiting, diarrho a
9. Cholesterol, free (S)	46.9	% of total	1.4	High in biliary obstruction
10. Cholesterol, total (S)	210	mg/100 ml	50	High in nephrosis
11. Copper (P)	114	μgm/100 ml	16	High in anaemia of infection
12. Creatinine (P)	1.0	mg/100 ml	0.15	High in renal insufficiency
13. Fat, total (P)	735	mg/100 ml	216	High in nephrosis
14. Fat, neutral (P)	225	mg/100 ml	137	High in nephrosis
15. Fat, phospholipid (P)	181	mg/100 ml	71	High in biliary obstruction
16. Fibrinogen (P)	0.2-0.4	g/100 ml	—	Low severe liver disease
17. Globulin (S)	2.0	g/10 ml	0.27	High in multiple myeloma
18. Glucose (B)	90	mg/100 ml	9.6	High in diabetes; low in steatorrhea
19. Haemoglobin, male (B)	15.9	g/100 ml	1.12	High in polychythemia; low in iron deficiency anaemia
20. Haemoglobin, female (B)	13.9	g/100 ml	0.86	Same as for male
21. Iodine, protein-bound (S)	5.0	μ gm/100 ml	0.68	High in hyperthyroidism; low in myxedema
22. Iron (S)	105	μ gm/100 ml	30	Low in iron deficiency anaemia; infection
23. Iron-binding capacity (S)	200	μ gm/Fe/100 ml	—	High in iron deficiency anaemia; low in infection
24. Ketone bodies, as acetone (B)	0.2-0.7	mg/100 ml	—	High in diabetes, starvation
25. Lactic Acid (B)	11.5	mg/100 ml	30	High in exercise
26. Nitrogen, non-protein (B)	29	mg N/100 ml	4.4	High in renal insufficiency

TABLE 3.5 (continued)

Constituent	Mean	Units	Standard deviation	Examples of abnormal value
27. O₂ content, arterial (B)	19.6	ml/100 ml	1.2	High in polycythemia; low in emphysema
28. O₂ content, venous (B)	12.6	ml/100 ml	1.3	Same as for arterial
29. pH (S)	7.36	pH Units	0.034	Low in diabetic acidosis
30. Phosphatase, acid (S)	2.8	Gutman Units	0.6	High in prostate carcinoma
31. Phosphatase, alkaline (S)	2.6	Bodansky Units	0.59	High in bone diseases with osteoblastic activity
32. Protein, total (S)	7.2	g/100 ml	0.35	High in multiple myeloma; low in nephrosis
33. Phosphorus (S)	3.6	mg/100 ml	0.42	High in hypoparathyroidism low in rickets
34. Potassium (P)	4.26	mEq/litre	0.43	High in adrenal insufficiency
35. Pyruvic acid (B)	1.04	mg/100 ml	0.36	High in thiamine deficiency
36. Sodium (S)	140	mEq/litre	1.7	Low in adrenal insufficiency
37. Thiamine (B)	3.4	μ gm/100 ml	1.2	—
38. Urea nitrogen (B)	13.6	mgN/100 ml	3.3	High in renal insufficiency
39. Uric acid (S)	4.4	mg/100 ml	1.1	High in gout
40. Vit. A, male (P)	128	IU/100 ml	29	Low in Vit. A deficiency
41. Vit. A, female (P)	91	IU/100 ml	22	Same as in male
42. Volume, plasma	45.3	ml/kg	5.5	Low in shock
43. Volume, RBC	34.8	ml/kg	5.1	High in polycythemia; low in nutritional oedema
44. Volume, whole blood	80.1	ml/kg	10.5	Low in dehydration

(Source: Textbook of Biochemistry by B. Harrow and A. Mazur, 1958).

S=Serum; P=Plasma; B=Whole blood; RBC=Red blood cells; •
IU=International Unit.

According to Bodansky, a value is abnormal when it is different from the mean by 2 to 3 times the standard deviation and the degree of alteration will vary with the severity as well as the stage of the disease. The alteration is not confind only to the disease mentioned in Table 3.5, there may be many other diseases in changes will occur.

Somogyi amylase unit is mg reducing substance liberated from standard sodium chloride-starch mixture by 100 ml serum in 30 minutes at 40°C. Gutman acid phosphatase unit is mg phenol liberated at pH 5.0 from standard phenylphosphate-citrate mixture by 100 ml serum in 1 hour at 37°C. Bodansky alkaline phosphatase unit is mg inorganic phosphate liberated at alkaline pH from standard glycerophosphate-veronal mixture by 100 ml serum in 1 hour at 37°C. In children these values are higher.

Further Reading

Bailey, *Textbook of Histology* (London: Williams & Wilkins, 1971).

G.H. Bell, J.N. Davidson and D.E. Smith, *Textbook of Physiology and Biochemistry* (London: ELBS, 1972).

Samson Wright, *Applied Physiology* (London: ELBS, 1971).

E.S. West *et al.*, *Textbook of Biochemist.* (London: Collier-Macmillan, 1974).

B. Harrow and A. Mazur, *Textbook of Biochemistry* (New York: W.B. Saunders, 1958).

P.B. Hawk, B.L. Oser and W.H Summerson, *Practical Physiological Chemistry* (New York: McGraw-Hill, 1952).

E.J. King and I.D.P. Wootton, *Micro-Analysis in Medical Biochemistry* (London: Churchill, 1956).

H.A. Harper, *Review of Physiological Chemistry* (London: Lange, 1969).

The Liver: Its Functions and Tests

Introduction

The liver is the largest gland of the body. It has an exocrine function: the secretion of bile, which is conveyed to the intestine by a system of ducts. Prothrombin, serum albumin and lipoproteins are produced by the liver. It functions in the storage of carbohydrate foods and in releasing them into the blood at such times as they are needed by the body. The liver cells also store fats, proteins and vitamins. The liver is an organ of excretion—essential in the removal of waste products from the blood. From metabolic point of view, the liver is the most complex internal organ in the body.

The functions of the liver are numerous, characterized by their multiplicity and diversity. Even so the liver has no groups of cells cytologically specialized for the performance of one function or the other. It differs from other glands in several other structural features.

ANATOMICAL STRUCTURE

Immediately below the diaphragm, the liver is situated in the upper and right part of the abdominal cavity. The basic structure of the liver is the lobule, consisting of cords of cells extending outward from the portal triad, which contains the intralobular bile duct and the final small branches of the circulatory vessels—portal vein, hepatic artery, and lymphatics. A system of capillaries and open spaces (sinusoids) containing blood, spreads from the lobules to surround individual liver cords and to conduct blood to the central hepatic vein, by which blood leaves the liver. Every lobule is supplied well with a capillary net work originating from the portal vein. Each hepatic cell is thus provided with an adequate amount of blood.

The hepatic lobule, considered as the anatomical unit of structure of the liver, has two main constituents—an epithelial parenchyma and a system of anastomosing blood channels. The parenchyma is made up of hepatic

cells arranged in irregular, branching and interconnected plates. The sheets of cell often appear as cell cords and are designated as hepatic cords. The hepatic plates or laminae form the secretory portions of the gland and are analogous to the secretory tubules of other glands. The hepatic plates are arranged in a definite manner relative to the blood channels, forming partitions between them. Such an arrangement slows down blood flow through the liver and so facilitates the exchange of materials between the blood and the liver tissue.

Blood Supply

The blood supply of the liver is peculiar in that, in addition to the arterial supply and venous return, possessed by all organs, the liver receives venous blood in large quantities through the portal vein (about 70 per cent). There are thus two afferent vessels—the hepatic artery and the portal vein. The hepatic artery carries the arterial blood (30 per cent) and the portal vein venous blood (70 per cent) from the intestines and spleen.

Duct System

The external secretion, the bile, is conveyed to the duodenum by the duct system of the liver. The smallest branches of the duct system are the narrow, intralobular bile canaliculi which from a ramifying net work of channels between the cells of the hepatic plates.

Most of the canaliculi drain from the net work in the outer limiting plate of the lobule into small interlobular bile ducts—terminal bile ducts, cholangioles, found at the periphery of a lobule. The short connections between the hepatic cells and the interlobular bile ducts are referred to as the canals of Hering.

Each interlobular duct joins with others forming ogressively larger ducts lined by cuboidal or columnar epithelium. With the increase in size of the ducts, the epithelium becomes high columnar and the connective tissue layer thicker. The interlobular bile ducts always accompany the branches of the portal vein and the hepatic artery in the process of ramifications through the connective tissue septa.

The right and left hepatic ducts join to form the hepatic duct which becomes the common bile duct after its juncture with the cystic duct and conveys the bile to the duodenum. The extra hepatic ducts of the liver are the hepatic ducts, the cystic duct, and the common bile duct, in contrast with the intrahepatic duct system within the gland.

The hepatic plates are made of the hepatic cells which are polyhedral; each cell having a central nucleus with a distinct nuclear membrane and one or more prominent nucleoli. The mitochondria of the hepatic cells are spherical or rod-shaped, the Golgi apparatus occurs near the edge of the cell beneath the bile canaliculus or close to the nucleus. The cytoplasm contains angular clumps of basophilic material consisting chiefly of nucleoproteins. Areas of glycogen and fat droplets also exist in the cytoplasm.

Structural and functional differences divide the liver lobule into three zones—an inner, hepatic zone around the central vein; an outer, portal zone at the periphery of the lobule and an intermediate zone between the central and peripheral regions. The three zones have different mitochondria based on their respective functional activity. Two types of cells occur in the lining of the sinusoids they could probably be the variations of the same type of cell, the stellate cells of von Kupffer, usually called the Kupffer cells. They are phagocytic cells forming a part of the reticulo-endothelial system. They are however considered as an integral part of the liver because of their intimate relation to the function of that organ.

Foreign substances are readily ingested and stored in large amounts in the cytoplasm of the Kupffer cells, which thus act to free the blood stream of foreign particles. They are also active in fat metabolism and in the formation of bile pigment.

The Functions of the Liver

The functions of the liver may be classified in five major groups—circulatory, excretory, metabolic, protective and haematologic.

Circulatory Functions

Transfer of blood from portal to systemic circulation; activity of its reticulo endothelial system (Kupffer cells) in immune mechanism; storage of blood and regulation of blood volume. The liver stores glycogen, fat and probably proteins, vitamins A, B_{12} and other substances concerned in blood formation and regeneration.

Excretory Functions

The exocrine function of the liver is concerned with the production of bile, which is carried by the system of bile ducts into the duodenum. Bile is produced by the hepatic cells and is partly a secretion which plays an important role in the absorption of fats and partly an excretion carrying off waste products which are eliminated with the faeces. Bile acids, bile pigments, cholesterol lecithin, neutral fats and soaps, traces of urea, water and bile salts are the constituents of bile. Absorption of fats in the intestine is facilitated by bile salts acting as emulsifying agents. The liver is also concerned in the excretion of substances withdrawn from the blood by hepatic activity, such as, heavy metals, dyes like bromsulphalein and alkaline phosphatase.

Metabolic Functions

The liver is pre-eminently the central organ of metabolism. It is concerned in the metabolism of carbohydrate, fat protein, minerals and vitamins; and in heat production. The roles of liver glycogen and regulation of blood sugar in carbohydrate metabolism are of special importance.

Protective Functions and Detoxication

Foreign bodies are removed from the blood (phagocytosis) by the Kupffer cell activity. Detoxication is effected by conjugation, methylation, oxidation, and reduction in the liver. It is also concerned in the removal of ammonia from blood, particularly that ~bsorbed from the intestine by way of the portal vein. Liver is the chief si ° of deaminization of amino acids, with the production of urea as a by-pioduct.

Haematologic Functions

Haematologic functions pertain to haematopoiesis and coagulation. Liver is concerned with the formation of blood in the embryo and in the 'idults under some abnormal sfates, in the production of fibrinogen, prothrombin and heparin and in erythrocyte destruction.

PHYSIOLOGIC AND CHEMICAL BASIS FOR TESTS OF LIVER FUNCTIONS

The liver performs many diverse functions and may tests have been devised to determine its functions. The results produced depend on the physiological reserve of the liver tissue, on the regenerative power of the liver and on its nutritional st ite. The body contains liver tissues far in excess of the minimal amount necessary for normal physiological function. Bile salts and bile pigments are not retained in the blood or excreted in the urine even after removal of 80 per cent of the liver in the dog. Moreover the tests differ widely in sensitivity in various pathological processes. The less sensitive tests may indicate normal results even when only 15 per cent of the liver parenchyma is functioning.

Bile Pigment Metabolism

The bile pigments are derived from the break down of haemoglobin taking place elsewhere in the body than in the liver. Bilirubin and biliverdin are the two principal bile pigments-the former being the chief pigment in the bile of carnivora, including man and the latter is the principal pigment in the avian bile although only a small amount is present in human bile.

The bile pigments originate in the reticulo-endothelial cells and the Kupffer cells may pla, a part where the erythrocytes are destroyed. The protoporphyrin ring of haem derived from haemoglobin is opened in the course of erythrocyte destruction to form the bile pigment biliverdin.

The human serum contains normally 0.1 to 1.5 mg of bilirubin per l00 ml. One g of haemoglobin produces about 35 mg of bilirubin. Bilirubin produced in the reticuloendothelial tissue from the catabolism of haem, is carried to the liver where conjugation with glucuronic acid takes place to form bilirubin glucuronide. Being more soluble in aqueous medium, the bilirubi: conjugate is readily excreted into the intestine with the bile.

The intestinal bacteria by their metabolic activity successively reduce bilirubin ($C_{33}H_{36}O_6N_4$) to the ultimate product stercobilin ogen ($C_{33}H_{48}O_6N_4$)
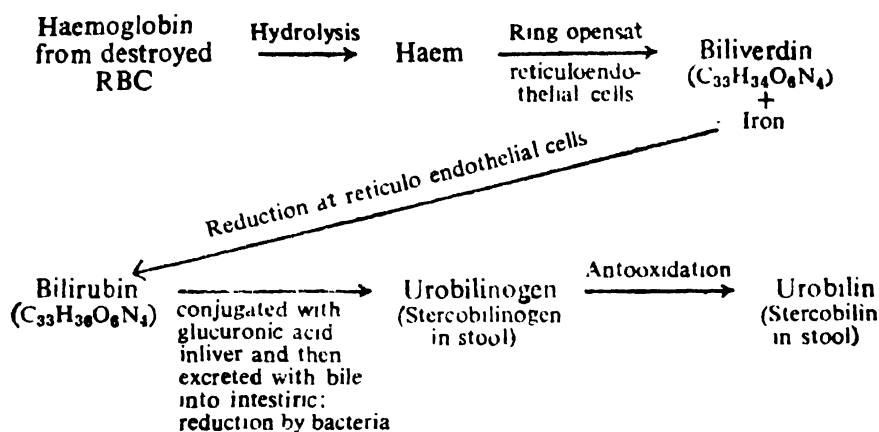
Haemoglobin   Hydrolysis   Ring opensat   Biliverdin
from destroyed ──────────►   Haem   ──────────────► $(C_{33}H_{34}O_6N_4)$
   RBC   reticuloendo-   +
                                       thelial cells   Iron

                                                        Reduction at reticulo endothelial cells

Bilirubin ◄──────────────►   Urobilinogen   Antooxidation   Urobilin
$(C_{33}H_{36}O_6N_4)$ conjugated with   (Stercobilinogen ──────────────► (Stercobilin
            glucuronic acid   in stool)   in stool)
            inliver and then
            excreted with bile
            into intestine:
            reduction by bacteria

Fig. 4.1   Bile pigment formation

which is also known as L-Urobilinogen. A portion of urobilinogen then
gets absorbed from the intestine into the blood, some of which is excreted
in the urine (1–4 mg per day, the remainder being re-excreted in the bile.
The faecal urobilinogen (40–280 mg per day) accounts for the unabsorbed
urobilinogen which is excreted in the stool.   Urobilinogen, on exposure to
air, is oxidized to urobilin, causing the stool to darken.

JAUNDICE

Bile pigment when present in excess in blood, passes into the tissues, which
turn yellow–a condition known as jaundice or icterus. Jaundice may
develop when the production of bile pigment is in excess to the capacity
of the normal liver to excrete. It may also arise from damaged liver
which fails to excrete the bilirubin produced in normal amounts. Jaundice
may also develop from the obstruction of the excretory ducts of the liver
which prevents the excretion of bilirubin.   The bile pigment concentration
in blood increases in all these conditions and beyond a certain limit
diffuses into ths tissues. Jaundice is thus classified into three groups
depending on the mode of its development—haemolytic, hepatic and
obstructive.

Haemolytic Jaundice
Haemolytic is caused when the destruction of erythrocytes is increased
with simultaneous increase in the formation of bile pigment. Normal
liver is unable to excrete the excess production of bilirubin, which rises
above the normal level in serum, resulting in haemolytic jaundice.

Hepatic Jaundice

Hepatic jaundice develops from damage to the parenchymal cells causing liver dysfunction. Liver poisons such as, chloroform, phosphorus, arsphenamine, carbon tetrachloride; toxins, hepatitis virus, engorgement of hepatic vessels in cardiac failure and cirrhosis are causative factors in the development of hepatic jaundice.

Obstructive (Regurgitation) Jaundice

Obstructive jaundice is caused from blockage of the hepatic or common bile ducts. Bile pigment probably passes from the blood, as usual, into the liver cells but the bile capillaries fail to excrete resulting in its absorption into the hepatic veins and lymphatics.

LIVER FUNCTION TESTS

The secretory and the excretory functions of the liver and the bile pigment metabolism constitute the liver function tests of major importance.

Estimation of Serum Bilirubin: Vanden Bergh Reaction

Vanden Bergh devised in 1913 a method for the quantitative estimation of serum bilirubin by the application of Ehrlich's test for bilirubin in urine (1883). The test is based on the coupling of diazotized sulphanic acid (Ehrlich's diazo reagent) and bilirubin to form a reddish-purple azo compound. For the reaction to take place alcohol was used as solvent in which both bilirubin and diazo reagent were soluble. When the reaction yielded the redding purple colour without alcohol it was termed as direct reacting, whereas for the colour developed after addition of alcohol the term indirect reacting was applied. The direct Vanden Bergh reaction is given when the bilirubin is conjugated in the liver with glucuronic acid (bilirubin glucuronide), which is soluble in water.

In obstructive jaundice, the conjugated bilirubin may return to the blood which gives the direct Vanden Bergh reaction. In the indirect Vanden Bergh reaction, the bilirubin is free (unconjugated with glucuronic acid) en route to the liver from the reticulo-endothelial tissues where bilirubin is formed by the breakdown of haem porphyrins. The free bilirubin is not soluble in water and therefore cannot react with the diazo reagent to produce the reddish-purple colour. The colour develops only on addition of alcohol. In haemolytic jaundice the increase in serum bilirubin is due to the indirect type—the free bilirubin.

A direct Vanden Bergh reaction with serum, where the bilirubin is conjugated with glucuronic acid, is generally associated with the appearance of bilirubin in urine. The colorimetric method of Malloy and Evelyn as modified by Ducci and Watson (1945) is used for the quantitative estimation of serum bilirubin. When the colour develops within one minute after addition of diluted serum (1 : 10 in distilled water) to the diazo reagent the presence of direct bilirubin is indicated which is indicated as

one-minute bilirubin. When the colour develops after thirty minutes on addition of diluted serum to a mixture of diazo reagent and methyl alcohol the total bilirubin content of serum is indicated. This is referred to as thirty-minute bilirubin.

Total bilirubin   = thirty-minute bilirubin
Direct (conjugated) bilirubin   = one-minute bilirubin
Indirect (unconjugated) bilirubin   = thirty-minute bilirubin one-minute
                                          bilirubin.

The colour is matched in a colorimeter against standard solutions of bilirubin and blank treated similarly using known amount of serum under test the normal bilirubin content of serum as given by Watson is given in Table 4.1.

TABLE 4.1   THE NORMAL BILIRUBIN CONTENT OF SERUM
(mg per 100 ml)

	Total	Direct	Indirect
Mean	0.62 ± 0.25	0.11 ± 0.05	0.51 ± 0.20
Upper limit of normal	1.50	0.25	1.25

Hyper-bilirubinemia may occur not only in diseases of the liver or biliary tract but also in disease states involving haemolysis such as in infectous diseases, pernicious anaemia, or haemorrhage. Repeated serum bilirubin determinations afford a means to follow the progress of manifest jaundice. A rising trend is an unfavourable sign. Improvement in the course of liver disease or of biliary obstructions is indicated by a progressive decline in serum bilirubin.

BILIRUBIN IN URINE

Hepatic parenchymatous or duct disease gives rise to elevated direct bilirubin concentration in blood making appearance of bilirubin in urine. A direct Vanden Bergh reaction in blood is accompanied by the presence of bilirubin in urine. Bilirubin in urine may be detected even before the clinical levels of jaundice are noted. Urine bilirubin is detected by the Harrison test, the Gmelin test or Huppert-Cole test, the first being the most sensitive.

Harrison Test
5 ml of urine and 5 ml of 10 per cent barium chloride solution are mixed

in a test tube.   Precipitate is collected on a filter paper which is dried
after spreading.   When dry, 1 or 2 drops of Fouchet's reagent (25 g of
trichloro-acetic acid 0.9 g of ferric chloride in 150 ml water) is added to
the precipitate.   A green colour indicates the presence of bilirubin in urine.

Gmelin Test
2 to 3 ml of urine is layered carefully over 5 ml of concentrated nitric acid
in a test tube.   Formation of rings of vario. s colours—green, blue, violet,
red, and reddish-yellow at the zone of contact, indicates the presence of
bilirubin in urine.

Huppert-Cole Test
5 ml of a suspension of calcium hydroxide in water are added to 10 ml of
urine, shaken well and filtered.   The bile pigment is removed with the
calcium hydroxide.   The residue on the filter is dissolved with 10 drops
of concentrated hydrochloric acid; the pigment set free is dissolved by
adding 10 ml of alcohol to the filter.   The alcoholic solution of the bile
pigments is collected in a tube and warmed on a water bath.   The presence
of bilirubin in the urine sample is detected by the development of a green
colour.

URINE UROBILINOGEN

Only traces (average 0.64 mg; normal upto 4 mg in   24 hours) of
urobilinogen are present in urine normally.   Urobilinogen is not found in
urine in complete obstruction of the bile duct due to the fact that bilirubin
is unable to get to the intestine to form it.   Presence of bilirubin in urine
without urobilinogen is suggestive of obstructive jaundice either intra-
hepatic or posthepatic.   In haemolytic jaundice, the increased production
of bilirubin leads to increased formation of urobilinogen, which appears in
urine in large amounts.   In haemolytic jaundice. bilirubin is not usually
found in urine.   Increased urobilinogen and absence of bilirubin in urine
indicate haemolytic jaundice.

Wallace-Diamond Test
This test determines urobilinogen in urine.   One ml of the aldehyde reagent
of Ehrlich (Solution of p-dimelhylaminobenzaldehyde acidified • with
hydrochloride acid) is added to 10 ml of urine in dilutions frqm 1:10 to
1 : 200.   The highest dilution showing a faint pink discolouration indicates
the urobilinogen concentration in urine.   Appearance of colour in
dilutions upto 1 : 20 is considered normal.   Persistence of colour in higher
dilutions is indicative of abnormal urobilinogen content in urine.

EXCRETION TESTS

The ability of the liver to remove a dye from the blood is determined by

the Bromsulphalein (BSP) excretion test. The test also indicates the efficiency of the liver in removing other substances from the blood which are normally excreted in the bile. The test consists in giving intravenous injection of 5 mg of brom sulphalein per kg body weight and blood sample is withdrawn after 30 to 45 minutes and the concentration of the dye in the plasma is estimated.

Less than 10 per cent dye is retained at 30 minutes and less than 6 per cent at 45 minutes. The BSP excretion test is a useful index of liver damage. The test is of no value in biliary obstruction.

Other excretory tests include Rose Bengal dye test, Bilirubin tolerance test. In Rose Bengal dye test, 10 ml of 1 per cent solution of the dye are injected intravenously. Normally 50 per cent or more of the injected dye disappears within 8 minutes.

Plasma Alkaline Phosphatase

Normal values of plasma alkaline phosphatase are 2 to 4.5 Bodansky units per 100 ml in adults and 3.5 to 11 in children. Alkaline phosphatase is normally excreted by the liver and obstructive jaundice causes a rise in the plasma alkaline phosphatase values. The values do not increase in purely haemolytic jaundice. Plasma alkaline phosphatase values also rise in other pathological conditions, such as, in rickets, hyperparathyroidism, Paget's disease, osteoblastic sarcoma and metastatic, carcinoma. Any biochemical test must correlate with the clinical findings and with other tests before a particular pathological condition is confirmed.

DETOXICATION TESTS

The protective function of the liver is based on a conjugation reaction by which benzoic acid is detoxicated by combination with glycine to form hippuric acid, which is excreted in the urine. The test can also be called as a metabolic function of the liver as the concentration and amount of glycine available will determine the rate of formation of hippuric acid.

$$C_6H_5COOH + H_2N-CH_2COOH \rightarrow C_6H_5CO-NH-CH_2COOH + H_2O$$
Benzoic acid Glycine   Hippuric acid

The test may be performed in two ways: (1) by intravenous injection of 1.77 g of sodium benzoate in 20 ml of water and collection of urine excreted during the hour after injection; (2) by oral administration of 6 g of sodium benzoate and a four-hour collection of urine.

Hippuric acid in the urine is isolated, hydrolysed and the benzoic acid produced is measured by quantitative titration. More than 0.7 g of hippuric acid is excreted in the one hour-urine after the intravenous injection under normal condition and the excretion of 3 g in the four-hour-urine in oral test is normal.

The presence or absence of intrinsic liver disease can be detected by

this test. Jaundice does not affect the test which therefore can be used to distinguish between intrahepatic and extrahepatic jaundice. Intrahepatic disease, such as, hepatitis or cirrhosis, causes a low output of hippuric acid. Cholecystitis, cholelithiasis and biliary obstruction from stones in the common duct give normal excretion of hippuric acid in urine. The test is of no value if renal function is impaired.

METABOLIC FUNCTION TESTS

Carbohydrate metabolism tests are based on the ability of the liver to remove sugars by glycogenesis or in the conversion of other sugars to glucose. Glucose tolerance test serves to evaluate the function of the liver in absence of other abnormalities in glucose metobolism.

Galactose Tolerance Test

Galactose, in common with various other sugars, like laevulose, xylose, is not metabolized directly in the body as in the case of glucose, but must first be transformed into glycogen. The capacity of the liver for transforming these other sugars into glycogen has been found to be altered in certain conditions of impairment of liver function and the rate of removal of the sugar in question from the blood forms the basis of an estimate of the capacity of the liver for transforming the non-glucose sugar.

The normal liver is able to convert galactose into glucose. intrahepatic disease brings about an impairment in this function of the liver with the result that the amount of galactose in the blood and urine remains excessive. The test, in conjunction with other investigations (bilirubin and alkaline phosphatase determinations in blood serum), may be useful in differentiating between obstructive and non-obstructive jaundice. Jaundice does not affect the test.

The test can be performed by intravenous injection of 0.5 g of galactose per kg of body weight given after a 12-hour fast. Blood galactose is estimated at various intervals. The 75-minute sample of blood does not contain any galactose under normal conditions but the value in intrahepatic jaundice in the 75-minute blood sample is greater than 20 mg per 100 ml and in obstructive jaundice it is present but less than 20 mg per 100 ml.

In the oral test 40 g of galactose in 500 ml of water is administered, followed by analysis of urine passed in the successive five hours to determine the total amount of galactose excreted. If the liver has metabolized the administered galactose to such an extent that less than 3 g is excreted in the five hours after the ingestion, its functional capacity is considered to be unimpaired. The excretion of more than 3 g of galactose is indicative of subnormal hepatic function. A more exact procedure is to estimate the rate of disappearance of galactose from the blood. The excretion amounts to 4 or 5 g or more in the five-hour urine in intrahepatic jaundice.

The *galactose time* is a convenient way of expressing the results of intravenous test.

$$\text{Galactose time (GT)} = \frac{a}{a-b} \times 90$$

a = galactose value in blood after $\frac{1}{2}$ hour of intravenous injection.

b = galactose value in blood after 2 hours of intravenous injection.

90 = time in minutes between the $\frac{1}{2}$-hour and 2-hour blood galactose values.

In normal subjects the galactose time is between 30 and 90, and may be elevated upto 184 in severe hepatitis and cirrhosis.*

Glycogen Storage: Epinephrine Tolerance Test

The elevation of blood sugar in response to epinephrine administration is a manifestation of hepatic glycogenolysis (breakdown of glycogen into glucose as main product in the liver and into pyruvate, lactate as main products in muscle). This is influenced directly by hepatic glycogen stores and the test therefore measures the glycogen storage capacity of the liver.

The subject is placed on a high carbohydrate diet for three days before the test. After an overnight fast, blood sugar is estimated and 0.01 ml of a 1:1000 solution of epinephrine per kg of body weight is injected. The blood sugar is estimated at 15-minute intervals upto 1 hour. The rise in blood sugar over the fasting level exceeds 40 mg per 100 ml under normal condition. The rise is less in hepatic disease. Diagnosis of glycogen storage disease (Von Gierke's disease) can be made with the help of this test.

Protein Metabolism

Fibrinogen, prothrombin, and albumin are mainly produced in the liver. Most of the α and β-globulins are also of hepatic origin, but the γ-globulins originate from plasma cells and lymphoid tissue. Dietary protein serves as a precursor of plasma protein. Normally, the approximate amounts of the proteins in plasma are albumin 4.0–5.7 g per 100 ml; globulin (excluding fibrinogen) 1.5–3.0 g per 100 ml; fibrinogen 0.1–0.5 g per 100 ml.

In pathological conditions, the albumin fraction in plasma is either unchanged or, more usually, lowered. Except in the presence of haemo-concentration or dehydration, the albumin fraction in plasma does not rise above normal. Prolonged malnutrition due to inadequate dietary intake of protein, impaired digestion of protein as in pancreatic insufficiency, or inadequate absorption from the intestine may produce a decline in plasma albumin (hypoalbuminemia). Chronic loss of protein, either in the urine as in the nephrotic syndrome or by extravasation as in burns

*S. Sherlock, *J. Path Bact.*, **58** (1946), p. 523.

may also result in hypoalbuminemia. A prominent feature of chronic liver disease. such as in cirrhosis, is the inability of the liver to synthesize albumin. The diagnostic and prognostic sign of this type of liver disease is found in hypoalbuminemia. Some types of hypoproteinemia are due to an inherited inability to synthesize plasma protein fractions (familial dysproteinemia) including the albumin or the globulins.

α and β-globulins undergo characteristic alterations under pathological conditions and are therefore of special interest in disease. All this indicate the importance of liver in the manufacture of albumin and other blood proteins. There is a general tendency to hypoproteinemia in acute and chronic liver disease. This is manifested mainly in the albumin fraction. A rise in globulin may occur in parenchymal liver damage. α_2 and β-globulins are characteristically changed in relapsing hepatitis. The severity of hypoalbuminemia in chronic liver disease is of diagnostic importance. A low plasma albumin which fails to rise during treatment is usually a poor prognostic sign.

Prothrombin Time

Dicumarol is used in the treatment of thrombosis, or intravascular clotting. Frequent determination of the prothrombin level of the blood are necessary. Warfarin has been used as an anticoagulant with considerable success in humans. The coagulability of blood is lowered in liver diseases, primarily owing to the lowered plasma prothrombin. A lowered plasma fibrinogen constitutes an additional contributory factor as a result haemorrhages may occur. Administration of Vitamin K fails to raise the abnormally low prothrombin level in such cases — the prolonged prothrombin clotting time. characteristic of liver disease, is unaffected.

The anticoagulants such as Dicumarol, Warfarin and other closely related compounds are used in selected types of surgery and in some heart conditions. Frequent determinations of the prothrombin level of the blood are necessary to ensure the efficacy of treatment and to control the dosage. This can be done by the method of Quick. An excess of thromboplastic substance, obtained from rabbit brain, and calcium are added to diluted plasma and the clotting time is noted. A clotting time is similarly noted with normal plasma which serves as control. By modification of the Quick method,* the prothrombin time can be determined in a one stage method. Normal levels of prothrombin in control sera give prothrombin times of about 15 seconds. Plasma prothrombin times may increase from 22 to as high as 150 seconds in impairment of liver function depending on the extent of its reduction. Severe liver damage is indicated by prolonged prothrombin time or by the occurrence of only a slight increase in prothrombin time after administration of Vitamin K. *This is due to the persistence of a prothrombin deficit.

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*A. J. Quick, *Am. J. Physiol.*, **118** (1937), p. 260.

Amino Acid Tolerance Test

The rates of disappearance of amino acids, administered orally or intravenously, from the blood may also serve to measure the protein metabolic function of the liver. Methionine and tyrosine have been found to be useful in this test. The rates of removal of these amino acids are retarded in liver disease.

Flocculation Tests

In liver disease there is a characteristic alteration in the distribution of the plasma proteins. Not only a reduction in serum albumin and a rise in serum globulin occur, but the relative magnitudes of the different components of the globulin fraction (α, β, γ) are changed. The results of the flocculation tests are related to these plasma changes. Various reagents are added to the serum under investigation. When the test is positive the added reagent is precipitated out of solution (flocculation) giving rise to turbidity. The flocculation is caused mainly due to the increase in the γ-globulin, which is commonly associated with hepatitis, although an increase in other globulins may play a minor part. The serum albumin inhibits flocculation. A number of tests to detect these proteins have been devised for clinical use.

The Cephalin-Cholesterol Flocculation Test (Hanger Test)

The test is made by mixing 4 ml of saline with 1 ml of cephalin-cholesterol emulsion and adding to this mixture 0.2 ml of serum. This is kept at room temperature in the dark without disturbance and the flocculations are read at 24 hours and 48 hours.

In normal serum the emulsion remains stable and flocculation does not take place. Varying degrees of both flocculation and precipitation occur under pathological conditions. The test is indicated in terms of $\pm$ to 4 + signs. 4 + indicates complete precipitation and flocculation; $\pm$ indicating more or less no change in the emulsion. Equivocal results ($\pm$ to 2 +) are considered negative. Active pathological process in the liver such as in acute hepatitis, can be diagnosed with the help of this test. In a case of currently inactive chronic liver disease with permanent liver damage the cephalin-cholesterol flocculation test may be negative with abnormal hippuric acid test. The progress of the infection in infectious hepatitis can also be measured with the help of the cephalin-cholesterol flocculation test. The test is positive in hepatic disease in portal cirrhosis and infective hepatitis.*

A normal liver function has been reported to be a prerequisite for the development and maintenance of essential hypertension.** The cephalin-cholesterol flocculation test is positive if: (1) gamma globulin is increased; (2) albumin of the plasma is decreased below the level which can inhibit

*S.K. Das Gupta *et al., Ind. Med. Gaz.,* 88 (1953) p. 8.
 S.K. Das Gupta *et al., Ind. Med. Gaz.,* 89 (1954) p. 4.
**S.K. Das Gupta *et al., Ind. Jour. Physiol and Pharmacol.,* 2 (April 1958).

the reaction; (3) the inhibiting ability of the plasma albumin is decreased.

Thymol Turbidity Test

0.1 ml of serum is added to 6 ml of a thymol solution and allowed to stand for 30 minutes. The turbidity is then read in a colorimeter against a barium sulphate standard. Normal is 0 to 4 units. Higher concentrations of beta and gamma globulins in serum give positive tests. The flocculation is noted after 18 hours and the results are indicated by ± to 4 + as in the case of cephalin-cholesterol flocculation test. Thymol turbidity and flocculation test correlates well with the cephalin-cholesterol test, although it is more, sensitive. Positive results are obtained in the thymol turbidity test for a longer period in the course of the disease than in the case of the cephalin-cholesterol flocculation test.

Thymol turbidity beyond 4 units with a flocculation greater than 1 + in 18 hours are considered abnormal. Thymol flocculation may be abnormal before turbidity records values higher than 4 units in early hepatitis. Thymol turbidity test does not depend on increased γ-globulin as is the case with the cephalin-cholesterol flocculation test. It requires lipids which are not necessary in the cephalin-cholesterol test. Conversely, gamma globulin which is necessary in cephalin-cholesterol test, is not involved in thymol turbidity. The flocculate in the thymol test is a complex lipo-thymoprotein. Positive results have been reported in liver diseases-hepatitis, cirrhosis, etc.* The thymol appears to decrease the dispersion and solubility of the lipids; and the protein is mainly beta globulin, although some gamma globulin is also precipitated.

Lipid Metabolism

The liver plays an important role in the metabolism of cholesterol, including its synthesis, esterification, oxidation and excretion. Total blood cholesterol under normal conditions, ranges between 150 to 250 mg per 100 ml, and about 60 to 70 per cent of the total cholesterol is esterified. The total blood cholesterol is increased along with the ester fraction (so that the esterified percentange does not change) in obstructive jaundice. In parenchymatous liver disease, the total cholesterol either remains normal or there may be even a decrease, the ester fraction being definitely reduced. The extent of decrease is roughly parallel to the degree of liver damage.

Brom Sulphalein Excretion Test

Normal liver cells secrete this dye (phenol and tetrabromphthalein disodium sulphonate) from the blood into the bile. Five mg of the dye per kg of body weight is injected intravenously and specimens of blood are collected after 5 and 45 minutes. The initial concentration of the dye in the blood is assumed to be 100 per cent. After 5 minutes the blood concentration normally falls to 85 per cent and after 45 minutes to 5 per

*S.K. Das Gupta *et al., Ind. Med. Gaz.*, **89** (1954), p. 12.

cent. A value at 45 minutes exceeding 10 per cent indicates liver damage.

Enzymes in Liver Disease

In liver disease the activity of a number of enzymes has been studied. The serum alkaline phosphatase may increase in obstructive hepatic disease as the enzyme is normally excreted by the liver. Other aspects of hepatic dysfunction may be reflected in the alterations in the serum concentrations of a number of other enzymes which are liberated from the liver as a result of the breakdown occurring in hepatocellular disease. High levels of serum lactic dehydrogenase (LDH) have occasionally been found in acute hepatitis during its clinical peak. Obstructive jaundice or metastatic disease in the liver gives rise to elevated serum lactic dehydrogenase content.

Serum glutamic-pyruvate transaminase (SGPT) as compared to serum glutamic-oxaloacetic transminase (SGOT) activity is relatively greater in liver than in other tissues. The measurement of SGPT has been found to be useful in the diagnosis and study of acute hepatic disease. SGPT is not significantly altered by acute cardiac necrosis, as is SGOT.

Acute hepatitis also record a marked increase in serum aldolase, and phosphohexose isomerase. No increase is found in cirrhosis, latent hepatitis, or biliary obstruction. The liver is a major, if not the only source of amylase in the serum under normal physiological conditions. In liver disease, therefore, serum amylase levels may be low. The activity of cholin esterase may also be lowered when disease involves the hepatic parenchyma.

The isocitric dehydrogenase (ICD) activity is increased in the early stages of hepatitis of viral origin. There is lesser elevation of the activity of this enzyme in serum in some malignancies with metastases to the liver. Cirrhosis of the liver and extrahepatic obstructive jaundice give ICD levels well within the normal range. No change is found in the activity of this enzyme in a number of other diseases including myocardial infraction.

α-hydroxy butyric acid dehydrogenase activity in serum has been found to be elevated in acute hepatitis and in myocardial infraction and has proved to be useful as a diagnostic aid. The ratio of the activities of serum lactic dehydrogenase to hydroxy butyric acid dehydrogenase with values over 1 : 5 is strongly suggestive of acute liver disease.

Excretion of Porphyrins

There is a marked rise of coproporphyrin excretion in urine in liver disease. Type I coproporphyrin increases in viral hepatitis in alcoholic cirrhosis it is type III. Uroporphyrin and porphobilinogen may appear in urine of patients with liver disease.

Vitamin Metabolism

The liver brings about the conversion of carotene to vitamin A and stores both vitamins A and D. Absorption of all the fat-soluble vitamins depends on the supply of bile to the intestine. Some abnormalities in the

metabolism of fat-soluble vitamins occur in obstructive jaundice. Vitamin A concentration in the blood is reduced. A lowered serum calcium occurs in obstructive jaundice. This may be due to a vitamin D deficiency arising from the absence of bile in the intestine. Prothrombin deficiency occurs from a failure to absorb vitamin K in obstructive jaundice. Prothrombin deficiency may also result from hepatic insufficiency.

Iron Metabolism

Liver disease causes significant alterations in the concentration of iron in serum. Two to three fold increases may occur in cirrhosis. Serum iron falls below normal. Serum iron remains normal in biliary obstruction. Many pathological states are associated with hepatic damage and dysfunction. The diverse functions of the liver have been studied through a battery of tests, known as liver function tests. None of these tests can serve as an index of the general condition of the liver because each test measures only a limited part of liver function.

TABLE 4.2. NORMAL VALUES OF VARIOUS LIVER FUNCTION TESTS

Physiological basis for the test	Test	Normal range of values
1. Bile pigment metabolism	Urine bilirubin, Urobilinogen (urine), Faecal urobilinogen Serum bilirubin	None 1–4 mg/24 hours 50–250 mg/24 hours 0.05–0.50 mg/100 ml
2. Enzyme activity	Plasma alkaline phosphatase SGOT	2–4.5 Bodansky units 8–40 units
3. Cholesterol metabolism	Esterified Total cholesterol	60–75% of Total 100–250 mg/100 ml
4. Protein synthesis	Serum albumin ,, globulin Total serum proteins Serum thymol turbidity Cephalin-cholesterol flocculation	3.4–6.5 g/100 ml 2.0–3.5 g/100 ml 5.7–8.2 g/100 ml 0–4 units 0–1+
5. Dye excretion	Brom sulphalein (5 mg/kg)	< 10% (30 minutes); < 6% (45 minutes)

Further Reading

N.F. Maclagan, 'Diseases of the Liver and Biliary Tract', in R.H.S. Thompson and I.D.P. Wootton, ed., *Biochemical Disorders in Human Disease*, 3rd ed. (London: Churchill Livingstone, 1970).

S. Sherlock, *Diseases of the Liver and Biliary System* (London: Blackwell, 1964).   .

Shamson Wright, *Applied Physiology* (London: ELBS, 1971).

Bailey, *Textbook of Histology* (London: Williams and Wilkins, 1971).

Harold A. Harper, *Review of Physiological Chemistry* (London: Lange, 1969).

The Kidney and Its Functions

Introduction

The kidney is the chief organ of the body for the elimination of water and a number of low molecular weight compounds dissolved in blood. The two kidneys, each weighing about 150 g in human adult, are located behind the peritoneum on either side of the vertebral column. Their position in relation to the vertebral column, however, varies with posture and respiration. The kidneys in good health are 11 to 13 cm in length, the left being as a rule the larger.

It is a compound tubular gland which separates urea and other nitrogenous waste products from the blood. The kidney maintains the constituents of blood plasma at proper levels. In doing so, it has an important role in regulating the chemical composition of the extracellular fluid which bathes the cells and tissues of the body. The chief functions of the organ might be to maintain the electrolyte composition of the body and to regulate its acid-base balance. Bilateral nephrectomy in animals or renal failure in man causes a progressive rise in the plasma concentration of urea, potassium ion and hydrogen ion leading to convulsions, coma and death within a few days. The function of the kidney in maintaining the composition of body fluids at a certain level is shared with the respiratory system, the skin, and the gastrointestinal tract.

About 1700 litres of blood are rinsed by the kidneys and about 170 litres of cell and protein-free filtrate are formed during 24 hours. To maintain a fluid of constant composition within the body, the kidney removes large quantities of both essential and waste materials from the blood; it then proceeds to salvage what the body requires, allowing the waste to be excreted as urine. The daily volume of urine may vary widely—according to whether the fluid intake is high or whether the fluid loss by external routes—notably sweating—is excessive.

STRUCTURE OF THE KIDNEY

.The longitudinal section of the kidney (Fig. 5.1) indicates the main gross anatomical features. The large central cavity, the renal pelvis, drains urine into the ureter. The pelvis extends into the renal tissue by prolongations known as calyces. The renal papillae, cones of tissue, project into the calyces. The tiny orifices of the ducts of Bellini open on to the papillae, the former being formed by the fusion of several collecting ducts. The renal tissue, especially at the papillary regions, is divided into an outer, dark cortex and an inner, pale medulla.

The blind-ended tubes called nephrons constitute the essential units of renal function. There are about a million nephrons in each human kidney.

Main features of Kidney in longitudinal section

Nephron

Proximal tubule

Distal tubule

Thin segment of Loop of Henle
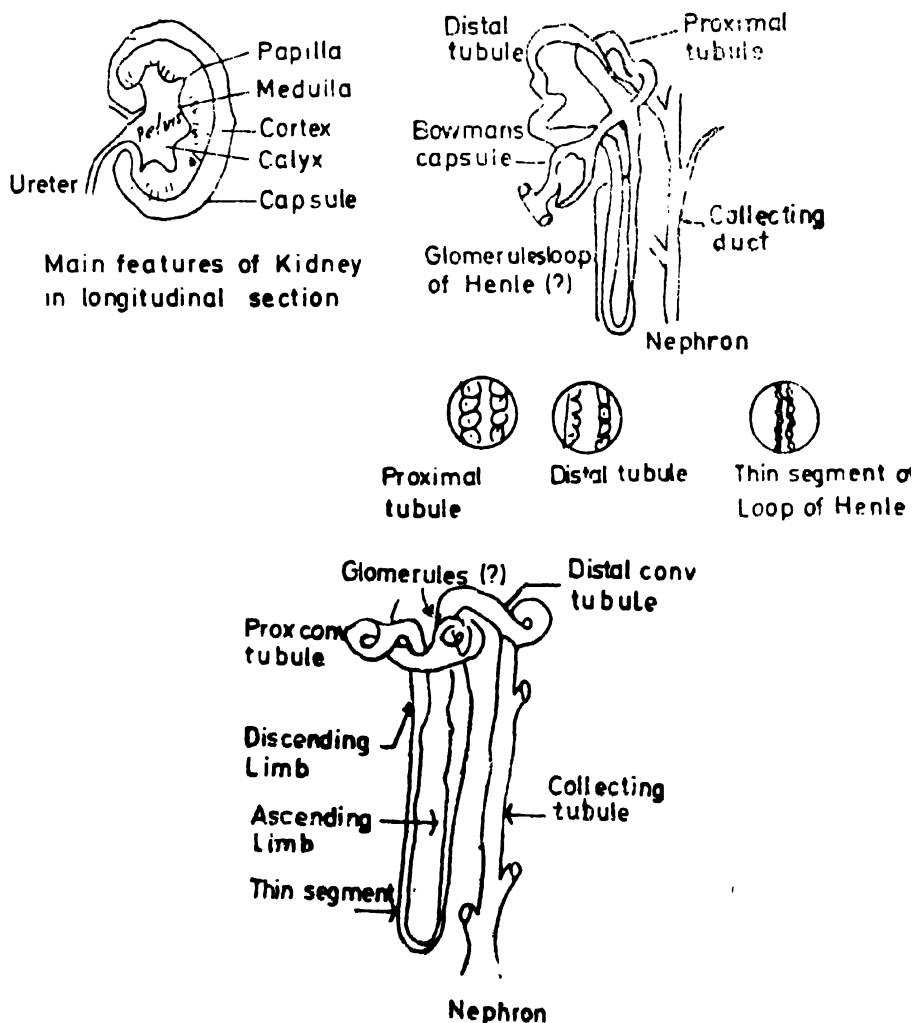
Nephron

Fig. 5.1

The nephrons consist of a single layer of epithelial cells surrounded by a basement membrane. A cup called the Bowman's capsule, occurs at the blind ends which lie in the cortex. The proximal tubule, a tortuous tube whose cells are well-developed with mitochondria and microvilli on the tubular surface, is immediately adjacent to the capsule. The straight part of the proximal tubule then dips down towards the medulla and ends when the cells become flatter and lumen narrower.

To each capsule is attached a long tubule including a convulated tubule terminating in the loop of Henle, and an ascending loop which ultimately connects with a main collecting tube opening into the renal pelvis. The straight parts of the proximal and distal tubules and the then U-loop are together known as the loop of Henle.

Nephrons may be divided into two types—the cortical nephrons and the juxta-medullary nephrons. Within each type there are variations in structure. Cortical nephrons have their capsules in the outer parts of the cortex. Their loops of Hanle are short, may not enter the medulla and may not even have a thin section. The juxta-medullary nephrons have capsules near the junction of medulla and cortex. They have long loops with thin sections which may reach almost to the tips of the papillae. Two or more distal tubules join to form collecting ducts which dip down through the cortex into the medulla. Finally, the collecting ducts join to form the ducts of Bellini.

The kidney represents a complex organ made up of innumerable small tubes, the uriniferous tubules. From each kidney a tube, the ureter, carries urine to the bladder and the urine is voided by means of another tube, the urethra.

Formation of Urine

The extracellular fluid constitutes the internal environment of the cells of the body. The cells carry out their vital activities in this medium. The internal environment is regulated by two pairs of organs—the lungs and the kidneys. The concentrations of oxygen and carbon dioxide are controlled by the lungs and the kidneys maintain the optimal chemical composition of the body fluids. The kidney does not merely remove metabolic wastes but actually performs highly important homeostatic functions. It has also a metabolic capacity.

The regulation of the internal environment by the kidneys involves four processes:

1. filtration of the blood plasma by the glomeruli;
2. selective reabsorption of materials needed for maintaining internal environment, by the tubules;
3. secretion by the tubules of certain substances from the blood into the tubular lumen for addition to the urine;

4. exchange of hydrogen ions and production of ammonia for conservation of base.

The urine is formed as a result to these four processes.

GLOMERULAR FILTRATION

The first stage in the formation of urine is filtration of non-protein substances across the glomerulus. A large volume of blood, 1 litre per minute, representing 25 per cent of the entire cardiac output at rest, flows through the kidneys. Thus the volume of blood that passes through the renal circulation in 4 to 5 minutes is equal to the total blood volume. All small molecules of the plasma can freely pass through the glomerular membrane, so also does the polysaccharide, inulin (M.wt. 5100). Purified egg albumin (M.wt. 40,000) also get through, serum albumin (M.wt. 68,000) cannot do so normally. The ability to pass through the membrane, to some extent. depends on the nature of the particle.

The energy for filtration is derived from the hydrostatic pressure of the blood and the filtration process occurs when the blood pressure in the glomeruli is greater than the sum of the osmotic pressure of the plasma proteins and the pressure in the Bowman's capsule. This is referred to as the filtration pressure. The mean arterial pressure in man is 100 mm Hg, and the glomerular capillary pressure is about 70 per cent of the mean arterial pressure, i.e., 70 mm Hg. The osmotic pressure of plasma proteins and the intracapsular pressure, opposing the filtration are about 30 and 15 mm Hg respectively. Thus the effective filtration pressure in the glomerulus is $70-(30+15)=25$ mg Hg. The quantity of filtrate produced by the glomerulus is governed by the effective filtration pressure and the amount of blood flowing through the kidneys. Normally these factors are maintained relatively constant by compensatory adjustments.

Renal filtration is reduced if the blood pressure fall is too severe for compensation. Thus a fall in aortic systolic pressure to 64 mm Hg will result in a pressure of about 45 mm Hg in the glomerular capillaries. This would mean the effective filtration pressure as zero and filtration would cease. Urine would not be formed (anuria) until the blood pressure is restored.

Ordinarily obstruction of a ureter causes cessation of urine formation when the pressure in the ureter is around 20 mm. The glomerular membrane fails to function as a filter for the blood when it is injured by disease. The capillaries may be completely occluded and thus removed from the active circulation. Blood cells and plasma proteins will leak through the injured capillary and will be excreted in the urine as it happens in glomerulonephritis.

The glomerular filtration rate (GFR) may be altered due to various nonphysiological factors. Occlusion of afferent arterioles by emboli (clot), ncreased intracapsular pressure due to obstruction in the tubules and larger

urinary channels, and decreased premeability of the glomerular membrane due to infection may cause a decline in the filtration rate. Increased blood pressure, decreased blood protein osmotic pressure and increased glomerular permeability will bring about an increased filtration rate.

Glomerular Filtration Rate (GFR)

Two million nephrons of both kidneys filter about one litre of blood each minute in the normal adult. 120 ml of glomerular filtrate are formed at Bowman's capsule at an effective filtration pressure of 25 mm Hg. The glomerular filtration rate (GFR) in adults is therefore about 120 ml per minute. Chemically, glomerular filtrate is essentially protein free extracellular fluid or a protein- and cell-free filtrate of whole blood.

TUBULAR REABSORPTION

The quantity of glomerular fluid formed in the kidneys is enormously greater than the amount of urine excreted. The glomeruli (both kidneys) filter 170 litres of solution per day and the volume of urine excreted in a day amounts to about 1.5 litres. 168.5 litres or 99 per cent of the glomerular filtrate, is reabsorbed as the fluid passes through the tubules.

The glomeruli function only as a filter and the composition of the glomerular filtrate is determined solely by the permeability of the capillary membrane to the constituents of blood. The glomerular filtrate thus contains may substances necessary for normal metabolism, such as water, glucose, amino acids and chlorides, as well as substances to be removed, such as urea, creatinine and uric acid. Essential substances are retained in greater or lesser amounts, under various conditions in accordance with the need to maintain constancy in the internal environment. The tubule carries out this highly selective function of the kidney. When a substance is moved from the tubular fluid to blood, it is said to be reabsorbed. If it is moved in the reverse direction, it is secreted. By reabsorption and secretion it modifies the glomerular filtrate and thus produces the urine.

About 80 to 87 per cent of the glomerular filtrate is reabsorbed along the upper (proximal) portion of the tubule—this fraction is referred to as 'obligatory' reabsorption. The urine remains isosmotic with blood plasma along this section of the tubule. Concentration of urine takes place along the lower (distal) portion of the tubule—this fraction is called 'facultative' absorption, which amounts to 13 to 20 per cent of the total filtrate. It is reabsorbed against the osmotic pressure of urine, and it requires energy. The metabolic activity of the renal tubular cells provides this energy.

The reabsorption of water is indicated by the higher concentration of dissolved substances in urine as compared to that in plasma and glomerular filtrate. The solute concentration in plasma and glomerular fluid is about 0.3 osmolar per litre, in urine it may rise five fold to 1.4 osmolar.

The *p*H of glomerular filtrate is about the same as that of plasma, 7.4.

As the glomerular filtrate passes through the proximal tubules, 80–87 per cent of the electrolytes, Na^+, K^+, Cl^-, HCO_3^-, HPO_4^{--}, and SO_4^{--}, and of water and practically all of the glucose, amino acids, and ascorbic acid are reabsorbed. The remaining 20 per cent passes to the distal tubules.

The increase in plasma volume due to water intake, is accompanied by increased cardiac output and glomerular filtration rate so that the proximal tubules absorb smaller amount of water than normal, and more passes on to the distal tubules. The reverse process also occurs—less water comes to the distal tubules, in dehydration and excessive concentration of blood plasma and other body fluids. Thus, the proximal tubules permit more water to pass when the plasma is too dilute and less to pass when the plasma is too concentrated.

The formation of urine is completed as the fluid from the proximal tubules passes through the loops of Henle, distal tubules, and collecting ducts where the cells reabsorb most of the water, various nonelectrolytes, and especially sodium salts from the fluid adding such substances as K^+, H^+, NH_4^+, and creatinine to it. Out of 20–25 ml of fluid reaching the loops of Hanle per minute, only 0.5 to 2.0 ml enters the collecting ducts.

Hormonal Control

The adrenal cortical hormones, aldosterone and deoxycorticosterone, control the reabsorption of electrolytes, chiefly sodium salts by the distal tubular and collecting duct cells. The adrenal cortex secretes the hormones in larger amounts which increase absorption of sodium salts when the electrolyte concentration and the osmotic pressure of plasma fall below a certain level. The increased activity of the adrenal cortex thus restores a larger proportion of electrolyte to the plasma and relieves the lowered electrolyte concentration and osmotic pressure. The adrenals secrete less hormones when the plasma electrolytes and osmotic pressure rise above normal. This permits the excretion of more sodium salts lowering the electrolyte concentration and osmotic pressure of plasma.

The secretion of cortical hormones may be greatly decreased in Addison's disease of the adrenals. This prevents the reabsorption of sodium salts and their loss in the urine causes profound disturbances of body fluid volume and composition.

The antidiuretic hormone (ADH), vasopressin is released into the blood by the neurohypophysis. This hormone controls the reabsorption of water by the distal tubules and collecting ducts. The neurohypophysis is stimulated to release more vasopressin when the electrolyte concentration and the osmotic pressure of plasma rise above normal. This causes more reabsorption of water back into the plasma with less water excretion in urine, to relieve the condition. Vasopressin is released into the blood in lesser amounts as the electrolyte concentration and osmotic pressure fall below normal. This lowers the water reabsorption with increasing excretion

in the urine. The secretion of vasopressin is deficient causing a decrease in the distal tubular reabsorption of water, in diabetes insipidus, with lesions of the hypophysis or hypothalamus. The excretion of urine under this condition is excessive, with very low specific gravity (1.002 to 1.006). This condition associated with great thirst and water intake, is controlled by administration of vasopressin preparations.

The ADH-controlled water reabsorption in the distal and collecting tubules is termed facultative reabsorption as it occurs independently of active solute transport and in accordance with the needs of the body for water. The obligatory reabsorption of water in the proximal tubule occurs secondary to solute reabsorption without regard for the water requirements of the body.

The net overall concentration of urine relative to glomerular filtrate is determined by the degree to which reabsorption of water exceeds reabsorption of total solutes in the glomerular filtrate.

Tubular reasbsorption of most substances of glomerular filtrate takes place as a result of operation of mechanisms, the so-called pumps, in the tubular cells which transport the substances against concentration or electrochemical gradients or against both. Transport of substances across the tubular membranes into the peritubular fluid against concentration and/or electrochemical gradients is called *active transport* as energy must be expended in the process by the transport mechanisms, most of which are located in the proximal tubules, although Na^+ also is actively transported in the distal tubules. The reabsorption of substances from the glomerular filtrate into the peritubular fluid involves many specific mechanisms. Some of these mechanisms reabsorb more than one substance. Glucose, xylose, fructose, and galactose are reabsorbed by a single mechanism. Reabsorption of cystine, ornithine, lysine, and arginine takes place by another mechanism

A few mechanisms can transport in both directions, thus promoting tubular reabsorption or secretion into the tubules. Two exchange mechanisms are known: one reabsorbs Na^+ and secretes K^+ and H^+ while the other reabsorbs Cl^- and secretes organic acid ions.

TUBULAR EXCRETION

In addition to a flow of substances from the plasma across the glomeruli, and a flow of substances across the tubular cells back into the plasma, there is also a flow from the plasma directly across the tubular cells into the lumen of the tubule. Thus many substances, both normal and foreign, are secreted into the tubules for excretion. Some are actively secretes substances such as carboxylic acids, phenol red, creatinine, *p*-aminohippurate, chlorothiazide, penicillin, and sulphuric acid esters. Another mechanism secretes strong organic bases like thiamine, choline, guanidine, and histamine. Secretion by these two mechanisms is localized in the proximal tubules. Secretion of K^+ and H^+ takes place in the distal tubules and collecting ducts.

Filtration, reabsorption and excretion of ions and water by man are indicated in Table 5.1.

TABLE 5.1

	Plasma concen- tration (meq/litre)	Glomerular filtration rate (litre 24 hrs)	Gibbs Donnan factor	Quantity filtered (meq 24 hrs)	Quantity excreted (meq 24 hrs)	Quantity reabsorbed (meq 24 hrs)	Per cent reabsorbed
Sodium	140	180	0.95	23,940	103	23,837	99.6
Chloride	105	180	1.05	19,845	103	19,742	99.5
Bicarbonate	27	180	1.05	5,103	2	5,101	99.9+
Potassium	4	180	0.95	684	51	633	92.6
Water	0.94 (litre/litre)	180	—	169.2 (litre/ 24 hrs)	1.5 (litre/ 24 hrs)	167.7 (litre/ 24 hrs)	99.1

From R. F. Pitts, *Physiology of the Kidney and Body Fluids* (London: Year Book Medical Publishers, 1963).

The product of glomerular filtration in litres per day, plasma concentration of the ion in meq per litre, and the appropriate Donnan factor for the ion represents the quantity of each ion filtered per day. Plasma contains about 6 g of protein per 100 ml (60 g per litre), with net negative charges and the glomerular filtrate is essentially protein-free. Thus the presence of nondiffiusible protein with negative charge and positively charged diffusible ions gives rise to a Donnan effect across the semipermeable glomerular membrane. This means that the concentrations of positive diffusible ions on the filtrate side of the membrane are less than those on the plasma side and the concentrations of negative diffusible ions are greater on the filtrate side. These facts account for the Gibbs-Donnan factors of 0.95 and 1.05 for the diffusible cations and anions, respectively.

ENERGY EXPENDITURE BY THE KIDNEY

For the formation of 1 to 1.5 litres of urine the kidney is required to expend 704 calories which corresponds to 70 g calories per gram of nitrogen, or 0.7 g calories per ml of urine. The kidney consumes energy of 6 to 11 calories per gram of nitrogen excreted for the production of this amount of urine. An efficiency of 1 to 2 per cent works out on the basis of the ratio of work performed and the energy used.

The total work performed by the human kidney in the formation of urine in 24 hours can be presented in Table 5.2.

<div align="center">

TABLE 5.2

Kind of work performed	Quantity of work $-\Delta F$) (g calories)
Concentration	−1126
Transport of water	+ 267
Formation of ammonia from urea	+ 155
Total	− 704

</div>

From Borsook and Winegarden, *Proc. Nat. Acad. Sci. U. S.,*
13, 1931, 17: 3(1931), p. 13.

Threshold Substances

Certain substances are reabsorbed almost completely by the tubule
provided their concentrations in the plasma are within the normal range.
When the concentrations of these substances in blood plasma exceed the
normal range, they make their appearance in the urine as they are not
completely reabsorbed. These are called the threshold substances. They
are glucose, ascorbic acid, Na^+, and Cl^-, which do not appear appreciably
in the urine until their plasma concentrations rise to certain values. The
glucose renal threshold ranges between 140 and 170 mg per 100 ml of
plasma. Glucose appears in urine when its plasma concentration rises
above its threshold value. A substance which is reabsorbed only slighly
or not at all is referred to as a low threshold substance such as, creatinine,
urea, and uric acid; on the other hand high threshold substances, which are
necessary to the body, are reabsorbed very efficiently (amino acids,
glucose, etc.).

REABSORPTION OF GLUCOSE

When the plasma concentration of glucose is normal it is completely
reabsorbed. Administration of the glucoside, phloridzin prevents its
absorption, inhibiting the oxidative process coupled with the phosphoryla-
tion of adenylic acid. Adenylic acid reacts with phosphate normally to
give ATP, which is the source of supply of energy to the mechanism,
actively concerned with the transport of glucose. The reabsorption of
glucose is paralysed whenever there is any interference with ATP
synthesis.
  • 120 mg of glucose are delivered into the glomerular filtrate each
minute when the arterial plasma level of glucose is 100 mg per 100 ml
and a glomerular filtration rate is 120 ml per minute. All of this glucose
is normally reabsorbed into the blood in the proximal convoluted tubule.
When the plasma glucose level is high, say, 200 mg per 100 ml and the
glomerular filtration rate remains unchanged, say, 120 ml per minute, the
amount of glucose delivered into the glomerular filtrate will be 240 mg
per minute for reabsorption. The capacity of the glucose transporting

system being limited, the reabsorption will continue until the full capacity of the tubular transfer system is reached and the excess which is filtered but cannot be reabsorbed will remain in the tubular fluid and pass into the urine. The excess glucose will carry water with it, resulting in the characteristic diuresis of glycosuria. The tubular maximum for glucose, referred to as TMG, denotes the maximum rate at which glucose can be reabsorbed. It is about 350 mg per minute. When the glomerular filtration rate is diminished, less glucose is delivered per minute for re-absorption. Plasma glucose will rise above normal (when glucose should have occured in urine) under this condition without glycosuria. It may be that under normal conditions the glucose-reabsorption-capacity of the tubule does not change, in diabetes mellitus and in hyperthyroidism however, it may be above normal.

Tubular defect in the reabsorption of glucose gives rise to the appearance of glucose in urine when the glucose level of plasma is normal. This reduction in the renal threshold of glucose is known as renal diabetes or renal glycosuria.

WATER REABSORPTION

It is estimated that Seven-eighths of the water which enters the proximal tubules from the glomeruli is reabsorbed ordinarily. Thus the glomerular filtration rate of about 100 ml per minute is reduced to about 20 ml per minute of a fluid which remains still isosmotic with the original glomerular filtrate. This happens due to the passive reabsorption of water as a solvent for the actively reabsorbed solutes like sodium chloride and glucose. This component of · water reabsorption is called obligatory reabsorption as it is secondary to the solute reabsorption regardless of the water requirements of the body in order to maintain the isosmotic environment in this area of the kidney. As it passes down the descending limb of the loop of Hanle, the tubular filtate becomes progressively more concentrated—hypertonic or hyperosmotic. Water without solute is lost from the descending limb of the loop of Hanle into the hypertonic environment, renal medulla and papilla which surround the loop. Active loss of sodium chloride without water occurs, however, in the ascending limb, so as to make the tubular filtrate upto the distal convoluted tubule hyposmotic. Antidiuretic hormone (ADH) acts at this point. There is little or no ADH activity if a dilute urine is to be excreted. On the contrary when conservation of water is required, the ADH activity increases which allows diffusion of water freely from the distal and collecting tubules, the urine becoming more concentrated, hyperosmotic, in the collecting tubules. The ADH-controlled water reabsorption in the distal and collecting tubules, is referred to as facultative reabsorption to indicate that it is independent of active solute transport and according to the needs of the body for water.

The ADH secretion is suppressed by a number of drugs and alcohol

resulting in increased urine flow. On the other hand excess production of ADH takes place under certain stresses like surgery, severe trauma or some drugs used in aneasthesia, resulting in excessive retention of water by the kidney—oliguria.

ELECTROLYTES REABSORPTION

The concentration of sodium does not alter throughout the proximal segment. Sodium is the principal cation and chloride and bicarbonate are the principal anions of the extracellular fluid and of the glomerular filtrate. These are selectively reabsorbed mainly in the proximal tubule. In normal conditions seven-eighths of the filtered Na^+ is actively reabsorbed (obligatory reabsorption) and the water follows passively. The osmotic pressure of the proximal tubular fluid throughout its course thus remains identical with that of the filtrate.

Sodium cannot be absorbed alone. It must be accompanied by anions. Of these Cl^- provides the greatest bulk and Cl^- is thus reabsorbed secondary to the absorption of sodium. In general reabsorption and excretion of Cl^- roughly parallel that of sodium.

The active transport of Na^+ across the cell involves movements of K^+ from the plasma. Potassium is present in small but important quantities in extracellular fluid and hence in glomerular filtrate. The clearance of potassium may exceed the glomerular filtration rate when potassium is excreted in large excess, indicating the excretion not only by filtration but also by tubular secretion. All of the potassium which is filtered is however later reabsorbed in the proximal tubule at normal rates of excretion. Tubular secretion of potassium in the distal tubules accounts for its appearance in the urine. The secretion of potassium by the renal tubule is closely associated with the hydrogen ion exchange and with the acid-base equilibrium.

The adrenal corticoids, particularly aldosterone, play an important role in the reabsorption of sodium and excretion of potassium. Addison's disease due to adrenocortical insufficiency is characterized by an excess loss of sodium with retention of potassium. Excess reabsorption of sodium and urinary loss of potassium however occur in hyperactivity of the adrenal cortex or by the administration of corticoid hormones. Adrenal tumours may also cause hyperactivity of the adrenal which is characterized by secretion of large amounts of aldosterone leading to a marked increase in sodium retention and to potassium loss. The normal clearance of potassium from 5 to 10 per cent of the GFR may rise to 40 per cent in aldosteronism. Severe dehydration, a decline in plasma volume and shock may occur in acute loss of sodium as in Addison's disease.

BICARBONATE REABSORPTION

The *p*H of the proximal tubular fluid remains much the same as that of

the filtrate despite the reabsorption of seven-eighths of the sodium and water filtered.

The metabolism of the tubular cell produces CO_2 which is acted on by carbonic anhydrase to form H_2CO_3. This ionizing into H^+ is secreted into the tubular fluid to combine with HCO_3^- which has been filtered by the glomerulus. H_2CO_3 thus produced yields in turn CO_2 and H_2O. CO_2 diffuses from the tubule fluid into the cell. Na^+ which is being actively reabsorbed by the tubule cell finally enters the blood along with HCO_3^-, formed by the cell metabolism. The HCO_3^- absorbed into the blood stream has thus not actually been absorbed from the tubular fluid, but the end result is the same, for the HCO_3^- filtered is absorbed as CO_2.

PHOSPHATE REABSORPTION

The concentration of phosphate in the glomerular filtrate is about 1 meq per litre, some 170 meq per litre of phosphate being filtered daily. About 40 meq per litre of phosphate are excreted in the urine. The reabsorption of phosphate takes place in the proximal tubules. Decreased capacity for the phosphate reabsorption is associated with changes in the metabolism of bone resulting from an excessive loss of phosphate from the body. Such changes are evident from a low phosphorus content and high alkaline phosphatase activity in the blood, Vitamin D-resistant rickets in children or idiopathic osteomalacia in adults (Milkman's syndrome) fall under such category.

ACID-BASE BALANCE

The hydrogen ion concentration of the plasma is maintained remarkably constant at pH 7.4 in the face of constant daily production of about 40 to 60 meq of H-ion, mainly by the incomplete oxidation of fats and carbohydrates and by the oxidation of sulphur containing amino acids. In abnormal metobolic conditions, such as in diabetic ketosis, as much as 400 meq of H-ion may be produced with a comparatively slight fall in plasma pH. In acidaemia the kidney is able to excrete an acid urine and a more alkaline urine in alkalaemia.

The ionic constituents of plasma are given in Table 5.3

TABLE 5.3

Cations		Anions	
	meq per litre of plasma		meq per litre of plasma
Na+	142	HCO₃⁻⁻	27
K+	5	Cl⁻	103
Ca++	5	HPO₄⁻⁻	2
Mg++	3	SO₄⁻⁻	1
	155	Org. acids	6
		Protein	16
			155

From Gamble, *Extracellular Fluid*, 1954.

The cations are referred to as fixed base and anions as fixed acid. Except for carbonic acid, which is regulated by the respiration, and the plasma proteins, all the other constituents are regulated by the kidney. The secretion of H-ions in the proximal tubule and the production of ammonia in the distal and collecting tubules determine the kidney's ability to vary the pH of the urine. The pH of normal urine varies from 5.0 to 7.0 with a mean of 6.0.

As the glomerular filtrate passes down the nephrons, its pH falls by 0.3 to 0.5 pH units in the proximal and distal tubule but in the collecting ducts by about 1 to 2 pH units. The secretion of H-ions occurs mainly in the proximal tubules but the volume c^f fluid in the collecting ducts is so small that a small addition of H-ion can bring about considerable changes in its pH.

The secretion of free H-ion in the proximal tubule is accompanied by the reabsorption of sodium bicarbonate from the tubular fluid under the influence of the enzyme carbonic anhydrase which increases the H-ion concentration in the tubule cell. The free H-ions pass into the tubular fluid in exchange for Na-ions which enter the tubule cell to be combined with the intracellular bicarbonate ion as sodium bicarbonate. This is then removed in the blood. Most of the H-ion combines with bicarbonate in the tubular lumen to form carbonic acid, which is then dehydrated to H_2O and CO_2. The CO_2 diffuses into the tubule cell and is either re-utilized to produce more H-ion or enters the blood. These exchanges result in the reabsorption of nearly all the bicarbonate of the glomerular filtrate with the H-ion becoming available for excretion. The H-ion is buffered by phosphate or combined with ammonia.
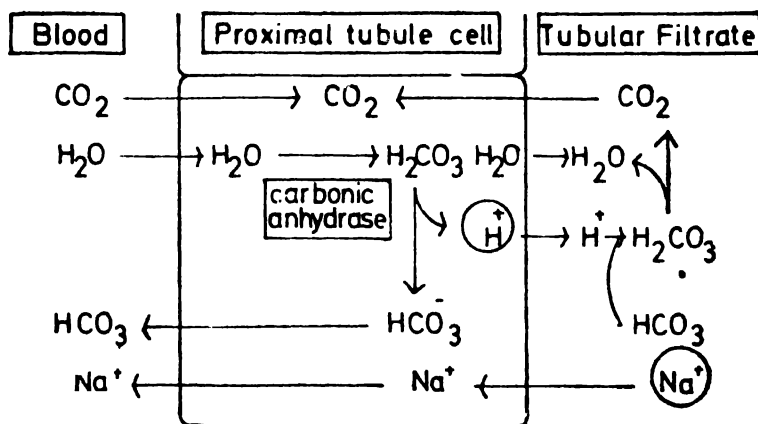
(a)

Fig. 5.2 (a)   Mobilization of H-ions in proximal tubule
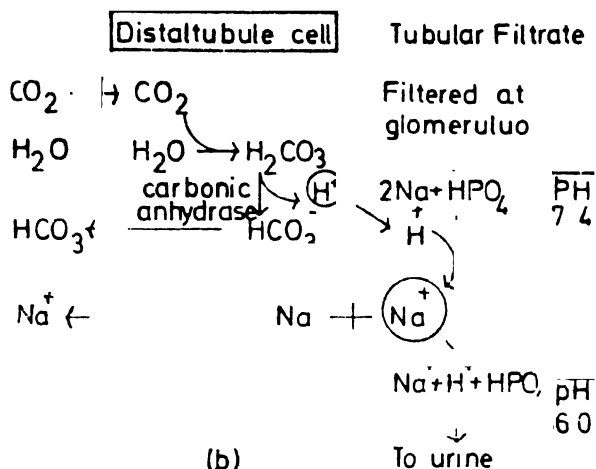
Fig. 5.2 (b)   Secretion of H-ions in distal tubule

The unabsorbed phosphate of the glomerular filtrate reacts as below:

$$HPO_4^{--} + H_2CO_3 = H_2PO_4^- + HCO_3^-$$
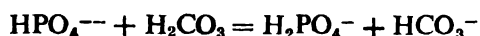
The dihydrogen phosphate is excreted and the bicarbonate reabsorbed to give a further supply of base to the plasma. The change from a pH 7.4 in the plasma to a pH 6.0 in urine is accomplished by a change in the proportion of the phosphates. In the plasma the ratio of $H_2PO_4^-$ to HPO_4^{--} is about 1 to 5 but in urine it is usually about 9 to 1. The ratio may rise to 50 to 1 when large amounts of H-ion are required to be eliminated.

Part of H-ion in the tubular fluid combines with ammonia formed from glutamine in the distal cells and collecting tubules by the action of enzyme glutaminase and from glutamic acid° by glutamate dehydrogenase. Ammonium ion is formed when the ammonia passing into the tubular lumen combines with free H-ion. NH_4^+ being a proton donor, is an acid.
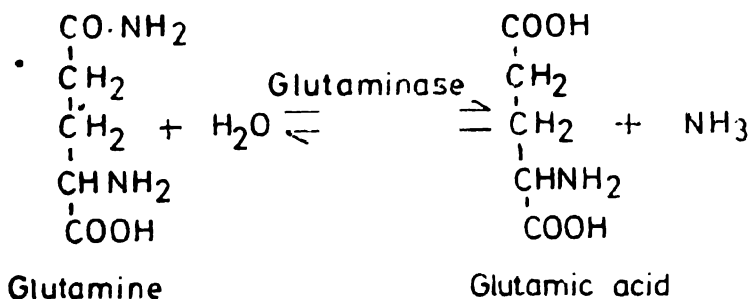
The buffering mechanism (mainly phosphate) and the ammonia mechanism share almost equally between them, the excretion of about

50 meq of H-ion per day in health.   The ammonia mechanism becomes more important under conditions when very large amounts of H-ion are required to be eliminated.   This is so because the ammonia mechanism can be increased about ten times.   The kidney however, is not the most important regulator of acid-base balance.   This is evident from the fact that 1500 meq of CO_2 is excreted through the lungs by a normal person at rest.

The amount of alkali required to raise the pH of urine to that of plasma determines the H-ion excretion by the kidney. This measures the ammonium excreted together with the titratable acid. v.   An adequate flow of urine is essential for the successful maintenance of acid-base equilibrium by the kidney.   This is ensured by giving large amounts of fluids in acidaemia and alkalaemia provided the kidneys are capable of response.

According to Pitts, the secretion•of acid by the tubles is due to an ionic éxchange.   The H-ion in the plasma, derived from H_2CO_3, is exchanged for Na-ion across the cell wall:
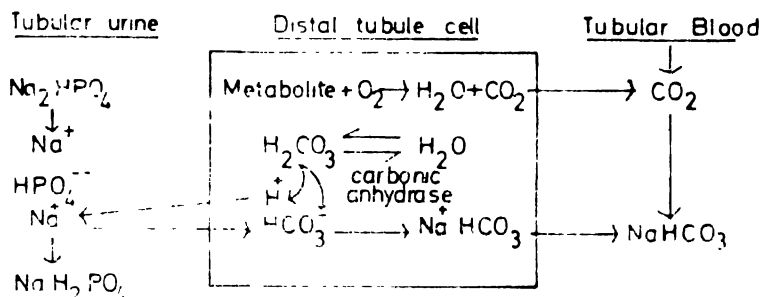
Fig. 5.3   Nature of the cellular mechanism for acidification of the urine
         (from Pitts and Alexander, *Federation Proceedings*, 7 (1948)
         p. 422)

RENAL FAILURE

Rapidly progressive irreversible disease processes, more commonly some acute disturbance to renal blood flow as in shock, may cause acute renal failure.   The production of urine falls below 400 ml per day (oliguria) and the quality of urine is also impaired.   The urine volume may fall to 20 to 30 ml per day and rarely, there may not be any urine at all (anuria). The concentration of nitrogenous products such as urea, creatinine in the blood rises rapidly, the plasma potassium increases and acidaemia develops. Unless the biochemical abnormalities are corrected, death may result in a week or two.

Progressive destruction of nephrons causes chronic renal failure resulting in acidaemia and gradual increase in blood urea and creatinine. The increase in blood urea and other nitrogenous substances in blood is proportional to the loss of nephrons, usually measured in terms of creatinine clearance.   The kidney has a very considerable reserve of

function and the blood urea seldom shows an increase until the creatinine clearance is less than half the normal value. When the creatinine clearance falls significantly, the surviving nephrons, subjected to greater load of material to be excreted (filtered load), are in a state of persistent osmotic diuresis and relatively large amounts diluted urine (polyuria) are excreted. The fractional excretion of electrolytes and water is increased. About 80 per cent of the fluid filtered and 50 to 60 per cent of the filtered load of sodium and chloride may be excreted at GFR of 3.0 ml per minute. The number of functioning nephrons fall a still further resulting in lowered urine volume and death from uraemia. The biochemical disturbances in uraemia involve acidaemia, retention of many nitrogenous end-products of which urea appears to be least harmful, and alterations in calcium, magnesium, potassium, and many other substances.

Severe renal failure cases may be treated by dialysis—a process by which some of the functions of the kidney are replaced temporarily. This may be done outside the body by connecting one of the patient's arteries to a cellophane tube, kept in a bath of dialysing solution, isotonic with blood containing physiological concentrations of electrolytes but no urea or nitrogenous substance. The other end of cellophane is connected to a vein. Urea and other nitrogenous substances diffuse from the blood into the dialysing fluid during this process of haemo-dialysis. The electrolyte concentrations on either side of the membrane are equalized. Transplantation of kidney from a healthy relative or from a cadaver, in replacement of damaged kidney, affords another means of treatment.

RELATION OF THE KIDNEYS TO HYPERTENSION

Arterial hypertension is one of the commonest diseases. Goldblatt discovered that persistent hypertension could be induced in the dog by constriction of both main renal arteries or by the constriction of one renal artery and the excision of the opposite kidney. Several experiments show that some active substance is discharged by the kidney into the blood. One of this consisted in transplanting a kidney to the neck, with no nervous connections with the rest of the body. A rise of blood pressure still occurred when the main artery to the kidney was constricted.

All this indicates that an active substance is released by the kidney into the systemic circulation. The substance responsible for this rise in blood pressure has been given the name renin. It is believed that renin, acting like an enzyme, decomposes serum globulin (hypertensionogen) to produce hypertensin, a polypeptide. This hypertensin is present in the blood of patients with malignant hypertension. Hypertensin is also known as angiotonin.

Different hypertensins have been obtained, depending upon the animal source of renin and of the α_2-globulin (hypertensinogen) upon which it acts. Hypertensin I was obtained from horse plasma by Skeggs and

associates.* Hypertensin-converting enzyme from horse plasma catalyses the conversion of hypertensin I to active hypertensin II, which is a octapeptide. The amino-acid sequence is indicated below:

$$\text{Asp} - \text{Arg} - \text{Val} - \text{Tyr} - \text{Ileu} - \text{His} - \text{Pro} - \text{Phe.}$$
$$\;\;1\qquad 2\qquad 3\qquad 4\qquad 5\qquad 6\qquad 7\qquad 8$$
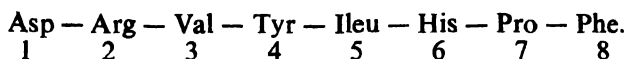
Hypertensin — 1 is decapeptide:

$$\text{Asp} - \text{Arg} - \text{Val} - \text{Tyr} - \text{Ileu} - \text{His} - \text{Pro} - \text{Phe} - \text{His} - \text{Leu.}$$
$$\;\;1\qquad 2\qquad 3\qquad 4\qquad 5\qquad 6\qquad 7\qquad 8\qquad 9\qquad 10$$
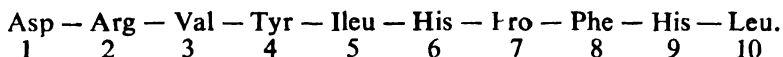
It appears that the plasma of •normal persons does not contain hypertensin II — the active hypertensin, but according to Skeggs the plasma of an individual with essential hypertension contained sufficient hypertensin II to maintain elevated blood pressure. Both the terms Angiotensins I and II are equivalent to hypertensins I and II respectively.

The rise of blood pressure is not a direct effect of renin; but renin acts as a proteolytic enzyme on plasma α-2-globulin substrate angiotensinogen to produce an inactive decapeptide angiotensin I from which angiotensin II is formed by the action of another enzyme. Agiotensin II is the most potent pressor substance known which causes generalized arteriolar constriction. It also stimulates the secretion of aldosterone from the zona glomerulosa of the adrenal gland promoting thereby sodium reabsorption by the kidney. Angiotensinase destroys angiotensin rapidily.

Plasma renin levels are usually expressed in ng Angiotensin II formed per 100 ml plasma in 3 hours' incubation at 37°C which is about 200 Mg per 100 ml normally.

The structure of the nephron and a comparison of the composition of the fluid reaching the kidney,• the blood plasma, and the fluid leaving it, the urine, constitute the physiology of the kidney. There is virtual absence* of protein and glucose in urine, the concentration of ammonia and creatinine in urine are very high when other substances such as sodium and calcium may appear nearly in the same concentration in urine and plasma.

Comparison of representative values for the concentration of certain substances in plasma and urine of man is shown in Table 5.4.

*L. T. Skeggs, Jr., J R. Kahn and N. P. Shumway, *J. Exp. Med.*, **103** (1956), pp. 295–301.

TABLE 5.4

Constituent	Plasma g per 100 ml	Urine g per 100 ml	Ratio of urinary concentration to plasma concentration
1. Water	90–93	95	—
2. Proteins and other colloids	7–8	0	—
3. Urea	0.03	2	60
4. Uric acid	0.003	2	15
5. Glucose	0.1	0	—
6. Creatinine	0.001	0.1	100
7. Ammonia	0.0001	0.05	500
8. Sodium	0.32	0.6	2
9. Potassium	0.02	0.15	7
10. Calcium	0.01	0.015	1.5
11. Magnesium	0.0025	0.01	4
12. Chloride as Cl	0.37	0.6	2
13. Phosphate, inorganic as P	0.003	0.12	40
14. Sulphate as H_2SO_4	0.003	0.18	60

Gamble shows the chief differences in the compositions of normal blood plasma and urine in Fig. 5.4.
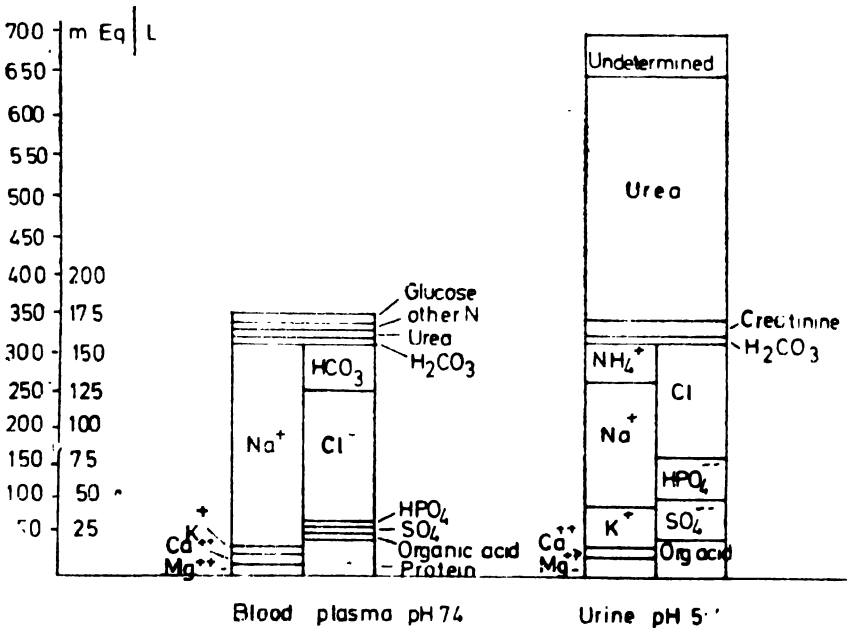
Fig. 5.4 (From Gamble, *Chemical Anatomy, Physiology and Pathology of Extracellular Fluid*, Cambridge, Mass: Harvard University Press, 1954).

The Volume and Composition of Urine

The volume and composition of urine even in health, varies widely from day to day being governed by, among other things, the type of food consumed, the volume of fluid taken and the amount of fluid loss by other channels.

Output

The daily output of urine is usually between 1000 and 1600 ml and the average amount of urine voided in 24 hours is about 1500 ml. The rate of production of urine is not the same in the 24 hours—the volume of urine during day is two to four times higher than that secreted during the night.

A continuous osmotic diuresis occurs in chronic renal disease. Increases beyond the normal amount (polyuria) occur in a number of diseases—chronic nephritis, diabetes insipidus, etc. Diarrhoea, fevers, etc., give rise to a reversed condition with elimination of a decreased quantity of urine—oliguria.

Specific Gravity

About 60 g of solids are present in 24 hours' urine—half of which is urea and one-quarter (or 15 g) is due to sodium chloride. Various organic and inorganic constituents such as uric acid, creatinine, amino acids, hormones, enzymes, vitamins, ammonia, sulphates, phosphates, etc., account for the remaining 15 g of solids. The specific gravity of urine in health varies between 1.001 and 1.040 but usually it ranges between 1.010 and 1.025 representing 40 to 60 g of dissolved solids in 24 hours.

Substances which are normally absent in urine u ke their appearance under pathological conditions. These include prote ns, sugar, acetone, bodies, bile, haemoglobin, etc.

Colour

The yellow colour of normal urine is mainly due to the pigment urochrome. The chemical nature and source of the substance are unknown. It is probably formed endogenously as its output increases with tissue protein destruction or increased metabolism. Small quantities of other pigments—urobilin or haematoporphyrin are also present normally.

The colour of urine may change considerably with the presence of abnormal constituents. Presence of haemoglobin gives rise to a brown to red colour. Bile in urine may produce a yellow foam when the sample is shaken with the colour becoming a pronounced brown.

Fresh urine is transparent. A cloud appears after some time. This may be due to separation of mucus, leukocytes, and epithelial cells. Phosphates, urates, pus, blood or bacteria may produce much cloudiness. On standing, normal urine becomes alkaline causing a precipitation of phosphates.

Odour
The volatile acids may be responsible for the development of the peculiar odour of urine. Urine undergoing decomposition has an ammonical odour. Certain dietary ingredients and a number of drugs give rise to sharp changes in odour. Urine gives out offensive odour with the intake of asparagus in the diet.

p*H*
The reaction of normal urine is usually acidic with a *p*H about 6. Even in normal conditions the variations may be considerable. The acidity of urine will tend to rise by an excess protein intake which produces increased quantities of sulphate and phosphate. Acidosis and fever also increase the acidity with a concentrated urine.

Urine on standing becomes alkaline due to the gradual conversion of urea into ammonia. This is not so with freshly voided urine. Alkaline urine may arise as a result of decomposition in the bladder—a decomposition of urea after the urine is secreted. On the other hand, a full meal or eating excessive quantities of fruit may give rise to a temporary alkaline tide. An alkaline ash results as the salts of organic acids present in fruits, are oxidized in the body. A normal individual will excrete an alkaline urine by ingestion of 3 to 5 g of sodium bicarbonate.

Urine on standing permits the organic substances present in it to undergo decomposition. Urea changes into ammonia and so on. It is therefore necessary to examine the urine fresh for the quantitative estimations of its constituents, failing which use is required to be made of preservatives such as boric acid, formalin, thymol, toluene, chloroform, etc.

Composition of normal urine alongwith range is indicated below:*

1.	Colour	Slightly yellow to amber
2.	Quantity	1000 to 1500 ml per 24 hrs
3.	Specific gravity	1.008 to 1.030
4.	*p*H	5.5 to 7.5
5.	Sugar	0.015 per cent
6.	Nitrogen Total	7 to 20 g per 24 hrs
	Amino acid	0.15 to 0.30 g per 24 hrs
7.	Urea	12 to 35 g per 24 hrs
8.	Ammonia	0.6 to 1.2 g per 24 hrs
9.	Creatinine	0.8 to 2.0 g per 24 hrs
10.	Uric acid	0.3 to 0.8 g per 24 hrs
11.	Hippuric acid	0.7 g per 24 hrs
12.	Chlorides as NaCl	10 to 15 g per 24 hrs
13.	Phosphorus (P)	1.2 g per 24 hrs
	Total	1.2 g per 24 hrs

*From Long, *Handbook of Chemistry*, 1949.

14. Sulphur (S) Inorganic : 1.0 g per 24 hrs
                  Ethereal : 0.1 g per 24 hrs
15. Sodium   : 2.5 to 4.0 g per 24 hrs
16. Calcium   : 0.1 to 0.3 g per 24 hrs
17. Potassium   : 1.5 to 2.0 g per 24 hrs
18. Magnesium   : 0.1 to 0.2 g per 24 hrs

Normal Constituents of Urine

Urea constitutes about one-half (25 g) of the total solids in urine and sodium chloride constitutes about one-fourth (10–15 g).

UREA

This substance represents the principal nitrogenous end product of protein metabolism in mammals. It is uric acid in birds and snakes and ammonia constitutes the principal nitrogenous end product in bony fishes.

The output of urea in man varies directly with protein intake and usually accounts for 80 to 90 per cent of the total nitrogen excretion. This ratio is lowered on a low protein diet. Increase in protein-catabolism causes increased urea excretion as in fever, diabetes or in excess adreno-cortical activity. Urea production is decreased in last stages of fatal liver disease resulting in its decreased excretion. In acidosis also urine urea is decreased as some of the nitrogen instead of being converted to urea is diverted in ammonia formation.

Urea is soluble in water and alcohol and insoluble in ether and chloroform. It forms biuret when heated, ammonia being eliminated:

$$H_2NCO - \overline{|NH_2 + H|} - {}^\bullet NH.CO.NH_2 \rightarrow H_2N.CO.NH.CONH_2 . + NH_3$$

In alkaline solution it is oxidized by hypobromite:

$$H_2NCONH_2 + 3NaOBr \rightarrow 3NaBr + N_2 + CO_2 + 2H_2O$$
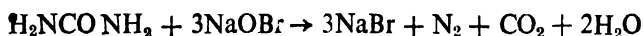
This reaction forms the basis for a rough quantitative estimation of urine urea by measuring the volume of nitrogen eliminated. The enzyme urease (present in soya and jack beans) quantitatively converts urea into ammonia providing a much more accurate method for its estimation. This is particularly used for estimating urea in blood.

Characteristic crystals of urea nitrate, $CO(NH_2)_2 . HNO_3$ and urea oxalate, $CO(NH_2)_2 . H_2C_2O_4$ are obtained by mixing urea with the respective acids. These salts are valuable for identification of urea.

AMMONIA

Freshly voided urine contains very little ammonia normally. In acidosis of

renal origin, the mechanism for urea formation by the kidney may fail resulting in low concentration of ammonia in urine. The ketosis and resultant acidosis of uncontrolled diabetes mellitus, in which renal function is unimpaired, brings about a high ammonia output in urine.

CREATININE AND CREATINE

Creatinine is the breakdown product of creatine:

$$
\begin{array}{ccc}
\underset{\text{Creatine}}{\begin{array}{l}\text{NH}_2\\ |\\ \text{C NH}\\ |\\ \text{N CH}_3\\ \text{CH}_2\text{COOH}\end{array}} \longrightarrow
\underset{\text{Phospho creatine}}{\text{HN}=\text{C}\begin{array}{l}\nearrow\text{NH PO(OH)}_2\\ \\ \searrow\text{N CH}_2\text{COOH}\\ |\\ \text{CH}_3\end{array}} \longrightarrow
\underset{\text{Creatinine}}{\text{HN}=\text{C}\begin{array}{l}\nearrow\text{NH}\\ \\ \searrow\text{N-CH}_2\text{-CO}\\ |\\ \text{CH}_3\end{array}}
\end{array}
$$
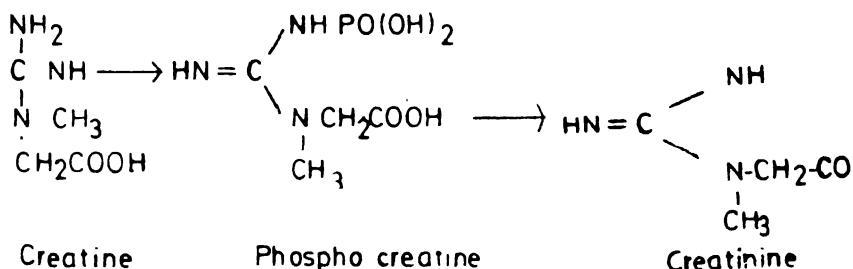
Creatinine is a normal constituent of urine and is relatively independent of the amount of protein in the diet. In many pathological conditions the urine creatinine is decreased. Creatinine is soluble in water and alcohol and forms a characteristic double salt with zinc chloride, $(C_4H_7N_3O)_2.ZnCl_2$. This serves as a means for isolating the base. A red colour is formed by the reaction of creatinine with picric acid in an alkaline solution, which is the basis for its colorimetric determination (Jaffe reaction). The reaction is due to the formation of a red tautomer of creatinine picrate. Creatinine also reacts with alkali and sodium nitroprusside to produce a red colour which turns yellow; on addition of acetic acid with heating the yellow solution turns green and finally a blue colour is produced (Salkowski reaction).

The creatinine coefficient is the ratio between the amounts of creatinine excreted in 24 hours to the body weight in kg. In normal men it is 20 to 26 mg/kg/day and 14 to 22 mg/kg/day in normal women. Creatine is present in the urine of children and in much smaller amounts in the urine of adults. In men the creatine excretion is about 6 per cent of total creatinine output. Creatine excretion is increased in pregnancy as well as in starvation, impaired carbohydrate metabolism, hyperthyroidism and in infections.

In hypothyroidism the creatine excretion is decreased. Creatine, when heated in acid solution, is converted into creatinine.

URIC ACID

This is the chief nitrogenous end-product in birds, snakes and lizards. It is derived from the nucleoproteins of the food and from the breakdown of nucleoprotein within the cells of the body. Uric acid is very slightly

soluble in water but forms soluble salts with alkali. From acid urine therefore it precipitates readily.

Uric acid forms salts with sodium and potassium to give the corresponding urates. The forms in which uric acid is largely found in urine. On strong acidification the free uric acid is obtained. The acid itself is also present to some extent. The urates, particularly the acid salts, are thrown out of solution when urine is concentrated, giving the sediment of amorphous urates.

The uric acid output is very much increased in leukemia, with destruction of leukocytes. This also happens in diseases of liver, an organ rich in nucleoprotein. Gout has long been popularly associated with a disturbed uric acid metabolism, but the connection is not well established. Before the attack of gout the output of uric acid is somewhat decreased but it is definitely increased for several days after the attack.

Murexide reaction is a characteristic test for uric acid. This is done by evaporating the uric acid with nitric acid and treating the residue with ammonia, a reddish violet product, murexide or ammonium purpurate, is obtained. The nitric acid oxidizes uric acid to dialuric acid and alloxan, which then condense:

Uricacid　　　　　Dialuric acid　　　Alloxan

Alloxantin

$\downarrow$ NH_3

Purpuric acid
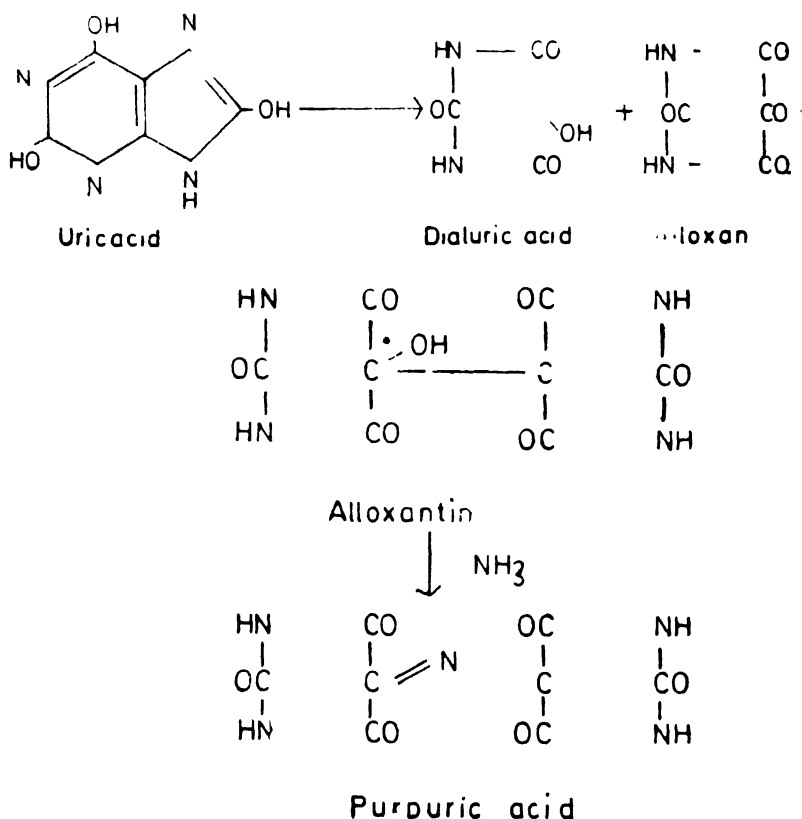
Uric acid reduces silver solutions in alkaline medium (Schiff test) and gives a blue colour with phosphotungstic acid (Folin) which serves as the basis for Folin colorimetric estimation.

The enzyme, uricase (from hog kidney) converts uric acid to allantoin:

Uric acid
(enol form)

Uric acid
(keto form)   Allantoin
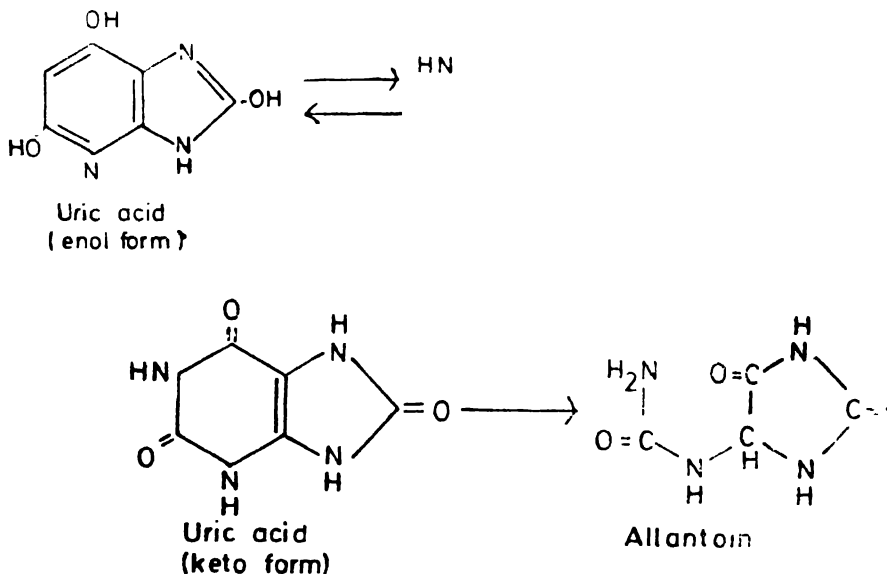
Allantoin is present in very small quantities in human urine but in other mammals it is the principal end-product of purine metabolism, replacing uric acid.

AMINO ACIDS

About 150 to 200 mg of amino acid nitrogen are excreted in 24 hours' urine of adults. The full-term infant at birth excretes about 3 mg amino acid nitrogen per pound body weight which declines gradually upto the age of six months when the value is 1 mg per pound. Premature babies excrete as much as ten times.

As the renal thresholds for these substances are quite high, very small amounts of amino acids are lost into the urine. All the naturally-occurring amino acids have been found in urine, some in relatively large quantities but most 'are present only in trace. A high percentage of some excreted amino acids is in combined forms and can be liberated by acid hydrolysis. Diet alters the pattern of amino acid excretion to a slight extent.

The use of protein hydrolysates in nutritional disturbances has made the study of amino acid excretion important. Methods based on micro-biological technique of paper chromatography are used extensively. Normal urine is usually protein-free, very small quantities are however present. On an average about 15 mg of albumin and 26 mg of globulin per 24 hours are found.

CHLORIDES

Next to urea, chlorides are the most abundant substances. The chlorides, mainly as sodium chloride, are derived chiefly from the food and the output, therefore, fluctuates, depending upon the intake. The output may be almost abolished during starvation and the chlorides in the blood will maintain for a time their normal concentration. The elimination of chlorides is decreased in several forms of nephritis and in fevers.

SULPHATES

The urine sulphur has its origin in protein because of the presence of the sulphur-containing amino acids, methionine and cystine, in the protein molecule. Much of it is derived from the protein in food and some of it has its source in cellular activity. The sulphur appears in the urine in three forms—inorganic sulphate, ethereal sulphate and neutral sulphur.

Inorganic Sulphates
The output of sulphate is proportional to the output of total nitrogen, the ratio $N : SO_3$ is about 5 . 1. Together with the total urinary nitrogen, this fraction of urine sulphur is an index of protein catabolism.

Ethereal Sulphates. (*Conjugated Sulphates*)
About nine-tenths of the total performed sulphates present in urine, is in the inorganic form, combined with Na, K, Ca, and Mg, and about one-tenth is in the form of ester, a combination of sulphuric acid with phenols:

OH

+ HO $\Big\rangle$ SO$_2$ $\longrightarrow$ O $-$ SO$_2$ $-$ OH
   HO

Phenol   Phenol Sulphuric acid
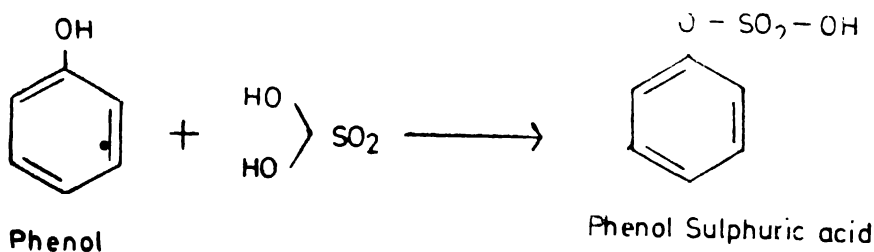
Other substances combined with the acid are *p*-cresol, indol (as indoxyl), and skatole (as skatoxyl). All these are called the ethereal sulphates. The ethereal sulphate fraction is largely derived from normal protein metabolism; but indican and some of the phenols result from the putrefactive activity in the intestine. Indican is the potassium salt of indoxyl sulphuric acid.

Diseases of the small intestine (intestinal obstruction) and in intestinal indigestion (biliousness) is characterized by indicanuria (substantial increase in indican output). Indican concentration increases in diseases of the

stomach in which there is subnormal amount of hydrochloric acid (gastritis and cancer).

The decomposition and oxidation of indican to indigo-blue (Jaffe, Obermayer) serve to detect indican:

Indoxyl

Indigo blue

The urine is mixed with ferric chloride and concentrated hydrochloric acid and chloroform is added and the mixture is shaken. The chloroform layer turns blue and the intensity of colour depends upon the amount of indican present. This is known as the Obermayer method for detection and estimation of indican in urine.

Inorganic sulphates are precipitated from urine by acidification with hydrochloric acid and addition of barium chloride. The precipitate is filtered off and the filtrate is heated when a second precipitate is formed due to the hydrolysis of the ethereal sulphate by the hot acid and the sulphate ion combining with the barium ion. This method serves as a means not only for the detection of the ethereal sulphate and the inorganic sulphate in urine but also as a basis for a quantitative estimation.

Neutral Sulphur

This is the sulphur which is in an incomplete state of oxidation possibly in the form of cystine, taurine, sulphides, thiocyanates, etc. This fraction is independent of the protein intake, like that of creatinine. The difference between the total sulphur and the sum of the inorganic and ethereal sulphur measures the neutral sulphur.

This type of sulphur is detected by treating the sample of urine with zinc and hydrochloric acid. Hydrogen liberated, combines with the sulphur to form hydrogen sulphide, which turns a strip of paper moistened with lead acetate into black due to formation of lead sulphide.

PHOSPHATES

The diet, particularly the protein content, influences the excretion of phosphates in the urine, but a small quantity has its origin in cellular metabolism. Two types of phosphates are known—the alkaline and the earthy phosphates. The alkaline phosphates account for two-thirds of the

whole and are salts of sodium and potassium whereas the calcium and magnesium salts constitute the earthy phosphates, which are precipitated in alkaline urine.

Urine on exposure for a time, gives rise to ammonia which combines with magnesium and phosphate to form ammonium magnesium phosphate, or triple phosphate. It is insoluble and is characterized by its crystalline structure. The output of phosphorus in urine is increased in certain bone diseases, such as osteomalacia and renal tubular rickets so also in hyper-parathyroidism. Phosphate excretion is decreased in hypoparathyroidism, in infectious diseases and in renal disease.

The two types of phosphates can be detected by precipitating the earthy phosphates first by ammonium hydroxide. The precipitate is filtered off and the filtrate is treated with magnesia mixture (magnesium sulphate + ammonium chloride + ammonium hydroxide) and warmed—a white precipitate of alkaline phosphate is formed.

Phosphate can be determined by addition of molybdate solution (sodium molybdate in sulphuric acid). Phosphomolybdate is formed which is then reduced by stannous chloride to form a blue compound; the intensity of blue colour measured colorimetrically, provides a method for its estimation quantitatively.

AMMONIA

The acid-base balance of the body is adjusted with the help of ammonia excreted as ammonium salts in the urine. The blood neutrality is endangered as acids use too much of the basic ions of the blood. This is prevented by the presence of ammonia as ammonium ions, which is excreted in increased amounts when acids or foods yielding acids in the body are fed. Its excretion, on the other hand, is decreased when alkali or foods yielding bases in the body are fed.

Normally amino acids are de-aminated and most of the resultant ammonia is converted into urea, only a small fraction escapes as ammonia. Some of the ammonia which normally would have been used in the formation of urea, is diverted to combine with acid radicals in order to prevent the danger of acidosis. This results in increased ammonia and decreased urea output.

One source of ammonia in urine is glutamine, an amino acid in the form of its amide. The glutamine content in blood is enough to account for much of the urinary ammonia formed by the conversion of glutamine to glutamic acid and ammonia—the remainder is derived from the amino acids of plasma. The conversion of glutamine to glutamic acid and ammonia is catalyzed by the enzyme, glutaminase, present in the kidney. This catalytic process is reversible under suitable conditions. The hydrolytic process is prevalent in the kidney whereas in other tissues the synthetic process is predominant.

Three de-aminating enzymes—glycine oxidase, D- and L-amino acid

oxidases, occur in the kidney indicating that deamination can also take place in the kidney. The ammonia in urine can be estimated by liberating it from its ammonium salts with addition of alkali and then aerating the liberated ammonia into an excess of standard acid. The un-neutralized acid is titrated with standard alkali.

ALLANTOIN

Partial oxidation of uric acid gives rise to allantoin. It occurs in small quantities in human urine, but allantoin is the chief end-product of purine metabolism in other mammals except the anthropoid ape. The chief end-product of purine metabolism in human being is uric acid.

OXALIC ACID

It occurs in urine as insoluble calcium oxalate, which is kept in solution by the presence of acid phosphate. It originates from ingested food such as cabbage, grapes, lettuce, tomatoes, etc. Ordinarily the amount of oxalate in urine is low.

CITRIC ACID

Though oxidized by the body easily, citric acid is nevertheless found in the urine. Alkalosis causes its increased excretion.

MINERALS

Four cations of the extracellular fluid, sodium, potassium, calcium, and magnesium, occur in the urine. Intake and physiological requirements cause considerable variations in the sodium content in the urine. Urine potassium rises with increased intake of in the presence of excessive tissue catabolism, diverting it from intracellular materials.

Acid-base equilibrium also affects potassium excretion. Alkalosis inevitably increases the excretion of potassium. The activity of the adrenal cortex also controls the excretion of sodium and potassium. The calcium and magnesium content in urine is relatively low as most of them are excreted by the intestine. Certain pathological states, particularly involving bone metabolism, cause a variation in the urinary excretion of calcium and magnesium in the urine.

VITAMINS, HORMONES, AND ENZYMES

Normal urine contains small quantities of these substances. Determination of these substances in the urine is often of diagnostic importance. Mention may be made of urinary ascorbic acid, urinary 17-hydroxy corticosteroids, 17-ketosteroids, and of urinary disease, etc.

Abnormal Constituents of Urine

A number of substances are found in the urine under pathological conditions—which are hardly found normal conditions. These are glucose, protein, acetone bodies, etc.

PROTEINS

A mixture of albumin and globulin in urine constitutes what is called proteinuria. Not more than 30 to 200 mg of protein are excreted in urine daily under normal conditions. In physiological proteinuria, less than 0.5 per cent of protein is present in urine. This may occur after severe exercise, high-protein diet or due to some temporary impairment in renal circulation arising as a result of standing erect. About 30 per cent of pregnancy cases exhibit proteinuria.

Pathological proteinuria may be classified as pre-renal, renal or post-renal as may be caused by factors operating before the kidney is reached, by the involvement of the kidney due to lesion intrinsic to the kidney or by inflammation in the lower urinary tract respectively. The degenerative phase of glomerulo-nephritis is characterized by proteinuria—the lowest excretion of albumin in urine occurs during the latent phase and may increase terminally.

In nephrosis a marked proteinuria occurs accompanied by edema and a low serum albumin concentration. The vascular form of renal disease, nephrosclerosis, is related to the problem of arterial hypertension. With the increasing severity of the renal lesion the proteinuria increases. The proteinuria is less in nephrosclerosis than that in glomerulonephritis. Patients suffering from arterial hypertension sometimes succumb to uremia—the toxic condition of urinary constituents in the blood. Poisoning of renal tubules by heavy metals like mercury, arsenic, or bismuth may also give rise to proteinuria.

The albumin may be detected by heating the urine when a white cloud or precipitate is formed which persists on addition of a little dilute acetic acid. In quantitative estimation of protein the urine is treated with trichloro acetic acid. The protein is precipitated and filtered and dissolved in sodium hydroxide and copper sulphate added. The intensity of colour formed is estimated colorimetrically (biuret).

Bence Jones proteins are globulins which occur in the urine most commonly in multiple myeloma (tumour like hyperplasia of the bone marrow) and rarely in leukemia, Hodgkin's disease, and lymphosarcoma. Bence Jones proteinuria is due to a peculiar protein in urine which precipitates at a low temperature, 50–60°C, dissolving to a greater or less extent when heated above 80°C, the precipitate appearing again on cooling.

The hydrolytic products of the protein indicate the presence of the common amino acids, except methionine. Since all the main fractions of

plasma protein, from which, presumably, the Bence Jones variety is derived, contain some methionine, the abnormal character of the Bence Jones variety becomes more pronounced.

GLUCOSE

Appreciable quantities of glucose in urine indicate glycosuria. Renal glycosuria is due to a lowered renal threshold. Glucose appears in the urine without any increase in blood glucose level. In diabetes there is an increase in blood glucose (hyperglycemia) with corresponding excretion of glucose in the urine (glycosuria). The glucose content in urine varys from 3 to 5 per cent or even higher. Presence of glucose in urine may be detected by the Benedict's test both qualitatively and quantitatively.

OTHER SUGARS

When the metabolism of fructose but not of other sugars, is disturbed, fructosuria may occur—although as a rare anomaly. *Galactosuria* and lactosuria may occur occasionally in infants and in mother during pregnancy, lactation and the weaning period. *Pentosuria* may occur after ingestion of large quantities of pentoses, such as plums, cherries, grapes, etc., congenital pentosuria is a benign genetic defect characterized by inability to metabolize, L-xylulose, a constituent of the uronic acid pathway.

All the sugars in urine reduce Benedict's solution. The sugars can be identified readily by paper .chromatography, or by the preparation of specific osazones.

KETONE BODIES

Acetone, acetoacetic acid and β-hydroxy butyric acid constitute the ketone bodies. These are present in traces in normal urine. In pathological conditions, they may increase from 0.02 to 6 g—β-hydroxybutyric acid often forming a large percentage. β-hydroxy butyric acid and acetoacetic acid, are eliminated as a salt, and so depletes the alkali reserve of the body, giving rise to an acidosis. More ammonia is formed in the kidney in order to meet the crisis. The acetone bodies in urine are increased in starvation, impaired carbohydrate metabolism, as in diabetes, pregnancy, ether anaesthesia and some types of alkalosis.

Acetoacetic acid decomposes readily into acetone. The qualitative tests for acetone bodies are as a rule, tests for acetone and acetoacetic acid. One of the tests is based on the conversion of acetone into iodoform. The urine is heated with sodium hydroxide and iodine and a precipitate of iodoform is formed with the characteristic odour. Ferric chloride gives a reddish colour with fresh urine, indicating the presence of acetoacetic acid.

To one-third test tube of solid ammonium sulphate are added not more than 5 ml of urine and 8–10 drops of sodium nitroprusside solution (0.25 per cent in 1 per cent nitric acid). After the addition of 1-2 ml of concentrated ammonia, the tube is shaken well and allowed to stand. A permanganate colour indicates the presence of acetoacetic acid. If the quantity is small the colour may develop slowly. This is called the Rothera's test and it detects, 1 part of acetoacetic acid in 100,000 parts of urine.

The nitroprusside test is for acetone, but acetoacetic acid decomposes so rapidly into acetone that it also gives this test. The ferric chloride test (Gerhardt's test) for acetoacetic acid is not given by acetone. The test for β-hydroxy butyric acid is seldom carried out, since it is somewhat involved, necessitating, first, the removal of the other two acetone bodies.

BILE

Jaundice or icterus develops as a result of the obstruction in the bile duct, preventing the normal outflow of bile and forcing it back into the general circulation. Bile pigments give rise to the yellowness of the skin and also appear in the urine. Play of colours obtained on addition of concentrated nitric acid to the urine indicates the presence of bile pigment, the various coloured products representing stages of oxidation of bilirubin (Gmelin test).

The urine containing bile gives a green colour when methylene blue is added. The bilirubin of bile and methylene blue react to form the green compound. The pigment related, chemically, to bilirubin and normally found in urine is urobilin.

BLOOD

Blood in urine may result (haematuria) from a lesion in the kidney or the urinary tract. This is more common than haemoglobinuria, in which haemoglobin without the red corpuscles is recognized. The liver is unable to change all of the haemoglobin into bile pigments where the destruction of the red blood cells is very great as in bad burns and some of the blood pigment appears in the urine. Free haemoglobin (haemoglobinuria) may also occur in the urine after rapid haemolysis such as in, blackwater fever.

PORPHYRINS

These iron-free pyrrole substances build haemoglobin and various oxidizing enzymes (cytochromes) under normal conditions. They form the building blocks for chlorophyll in the plant world together with magnesium. There are several varieties of porphyrins. One of them is coproporphyrin. It is

excreted under normal conditions, to the extent of 14 to 99 mg in the urine and 100 to 200 in faeces daily.

Porphyria is the metabolic disturbance involving the excretion of abnormal amounts of porphyrins. This may occur in cirrhosis, obstructive jaundice, etc. Hans Fischer was able to isolate two of the porphyrins—copro- and uroporphyrin from cases of congenital porphyria. These two differ in chemical structure from those found in the urine and faeces of normal subjects.

Kidney Function Tests

These are important for two reasons. They help, in the first place, to locate the site of impairment of renal function and secondly, in providing information concerning the normal biochemical function of the cells of the kidney.

Substances to be determined in blood and urine, may be normally present, such as urea or may be foreign to the body and intravenously injected. The result is expressed in terms of amounts of substance found in urine over a unit of time to the volume of plasma which it would occupy at the existing plasma concentration.

RENAL CLEARANCE

The concept of clearance has proved useful in estimating kidney function. The clearance value of a plasma constituent is the volume (in ml) of plasma which contains the amount of the constituent which is excreted in the urine in one minute. The term 'clearance' can be applied to any substance in the plasma which appears in the urine by whatever process it reaches the urine—filtration, secretion or excretion or a combination of these.

UREA CLEARANCE TEST

The normal blood urea is generally 25–40 mg per 100 ml. This level fluctuates in health with the protein intake. A rise in blood urea provides a sensitive index of renal failure. Blood creatine increases from normal value of 2 mg per cent to 3–5 mg/100 ml in renal failure, so also does uricacid. The urea clearance test aims to obtain a measure of the efficiency with which the kidney excretes urea. It shows the response of the kidney to the stimulation of the actual amount of urea in the blood. The clearance is defined in terms of the number of millilitres of blood cleared of urea per minute.

With large volumes of urine (above 2 ml per minute), the output of urea depends upon, and is directly proportional to, the level of urea in the blood. When the rate of urine excretion falls below 2 ml per minute this

direct relationship no longer holds. More of the urea is reabsorbed from the more highly concentrated urine in the tubules, and the clearance is therefore reduced. The output of urea is now found to vary not only with the blood urea, but also with the square root of the volume of urine.

In order to obtain a maximum physiological stimulation of the kidney, urea is given (15 g) to the patient with a liberal drink of water (500 ml) before commencing the test. Water alone is given in cases where the blood urea is already high. The necessary data are the urea concentration of the blood and the urine, and the volume of urine excreted in the given time.

The normal maximum clearance (when the flow of urine is above 2 ml per minute) is usually about 40 per cent greater than the standard clearance (when the flow of urine is less than 2 ml per minute). The average normal values for the maximum clearance are 75 ml of blood per minute with variations from 64 to 99 ml, and for standard clearance 54 ml with variations from 40 to 68 ml.

Nephritic patients with diminishing renal efficiency show a decreased clearance, down to about 40 per cent of the average normal value, before the blood urea and blood creatinine begin to rise; on the other hand the maximum specific gravity of the urine is often diminished before the urea clearance test gives abnormal results. When the clearance falls to 5 per cent the symptoms of uraemia usually appear.

Method
The patient is given no breakfast and nothing to drink in the morning other than water, if described.

0 hours	:	Bladder is emptied. 15 g of urea ı 500 ml of water, if blood urea is not raised (when only 500 ml of water is given)
½ hour	:	Blood is taken for estimation of blood urea (Blood No. I.)
1 hour	:	Bladder is emptied and complete urine specimen is collected (Urine No. I)
1½ hours	:	Blood is taken for estimation of blood urea (Blood No II)
2 hours	:	Bladder is emptied and the complete urine specimen is collected (Urine No. II)

The success of the test depends on collecting for analysis all the urine which has been excreted into the bladder during the stated time interval.

If the excretion of urine is at a rate greater than 2 ml per minute, the so-called maximum urea clearance is calculated according to the formula:

$$C_m = \frac{UV}{B}$$

C_m = Maximum clearance, i.e., the volume of blood cleared of urea in one minute during moderate diuresis

U = Urine urea concentration in mg per 100 ml

V = Volume of urine in ml per minute

B = Blood urea in mg per 100 ml

When the excretion of urine is less than 2 ml per minute, the so-called standard urea clearance is calculated from the formula:

$$C_s = \frac{U}{B} \sqrt{V}$$

C_s = Standard urea clearance

U, V, and B are the same as for C_m

The result of the test is usually expressed as a percentage of the average normal clearance, which is taken to be 75 ml of the blood per minute if the rate of urine excretion is above 2 ml per minute (C_m) and 54 ml blood if the rate is below 2 ml per minute (C_s). C_m is therefore multiplied by (100/75) or 1.33 and C_s by (100/54) or 1.85.

Examples

1. Urea clearance test on normal individuals:

	1st hour	*2nd hour*	*3rd hour*
Blood urea	65 mg/100 ml	75 mg/100 ml	72 mg/100 ml
Urine urea	1950 ,, ,,	3000 ,, ,,	3930 ,, ,,
Urine volume	121 ml	86 ml	35 ml
Clearance	$C_m = \frac{1950}{65} \times \frac{121}{60}$	$C_s = \frac{3000}{75} \sqrt{\frac{86}{60}}$	$C_s = \frac{3930}{72} \sqrt{\frac{35}{60}}$
Blood cleared of urea per minute	60.1 ml	48 ml	41.9 ml
Per cent of normal function	80 ($C_m \times 1.33$)	89 ($C_s \times 1.85$)	77 ($C_s \times 1.85$)

2. Urea clearance test in nephritis:

	1st hour	*2nd hour*
Blood urea	63 mg/100 ml	69 mg/100 ml
Urine urea	1275 ,, ,,	1770 ,, ,,
Urine volume	96 ml	70 ml
Clearance	$C_s = \frac{1275}{63} \sqrt{\frac{96}{60}}$	$C_s = \frac{1770}{69} \sqrt{\frac{70}{60}}$
Blood cleared of urea per minute	25.6 ml	27.7 ml
Per cent of normal function	$C_s \times 1.85 = 47$	$C_s \times 1.85 = 51$

3. Urea clearance in sub-acute nephritis:

	1st hour	2nd hour
Blood urea	88 mg/100 ml	102 mg/100 ml
Urine urea	900 ,, ,,	895 ,, ,,
Urine volume	116 ml	134 ml
Clearance	$C_s = \dfrac{900}{88}\sqrt{\dfrac{116}{60}}$	$C_m = \dfrac{895}{102} \times \dfrac{134}{60}$
Blood cleared of urea per minute	14.3 ml	19.6 ml
Per cent of normal function	$C_s \times 1.85 = 27$	$C_m \times 1.33 = 26$

4. Urea clearance test in uraemia:

	1st hour	2nd hour
Blood urea	209 mg/100 ml	209 mg/100 ml
Urine urea	615 ,, ,,	615 ,, ,,
Urine volume	100 ml	95 ml
Clearance	$C_s = \dfrac{615}{209}\sqrt{\dfrac{100}{60}}$	$C_s = \dfrac{615}{209}\sqrt{\dfrac{95}{60}}$
Blood cleared of urea per minute	3.8 ml	3.7 ml
Per cent of normal function	$C_s \times 1.85 = 7.0$	$C_s \times 1.85 = 6.9$

The term clearance value is somewhat misleading because no part of the plasma is completely cleared of urea. The determina·· ·n of the clearance value for certain substances provides a measure of ·he volume of the glomerular filtrate and of renal efficiency.

INULIN CLEARANCE—GLOMERULAR FILTRATION RATE (GFR)

The renal plasma clearance, UV/P (U = concentration of the substance in urine; V = volume of urine excreted per minute and P = its concentration in blood plasma), of a substance which is completely removed from the blood by the kidneys is equal to the renal plasma flow. No ,substance is consistently completely cleared, but at low plasma concentration of para aminohippuric acid (PAH), its clearance is between 85 and 100 per cent of the renal plasma flow, and is known as the effective renal plasma flow (ERPF). In the presence of gross tubule dysfunction PAH clearance is no longer a close approximation to renal plasma flow.

It is convenient to combine with the ERPF determination the determination of glomerular filtration rate (GFR). The soluble polysaccharide inulin is filtered out from the glomeruli in the same concentration as in plasma; in the tubules the inulin is neither reabsorbed nor excreted and

its clearance is equal to glomerular filtration. The fraction of plasma filtered at the glomerulus can be calculated:

$$\frac{GFR}{ERPF} = \text{Filtration Factor (FF).}$$

When the plasma inulin concentration (P) is 100 mg per 100 ml and the inulin excretion in the urine per minute (U_m) is 127 mg:

the clearance rate $(CR) = \dfrac{U_m \times 100}{P} = \dfrac{127 \times 100}{100} = 127 \text{ ml.}$

As inulin is neither reabsorbed nor excreted in the tubules, 127 ml of plasma could have been cleared of inulin by the filtration of 127 ml of (protein-free) plasma through the glomeruli. Inulin clearance value therefore is the glomerular filtrate volume.

Inulin is a polysaccharide which yields fructose on hydrolysis and has a molecular weight of 5200. The fructose is determined by the Seliwanoff reaction, in which it forms a cherry-red colour with resorcinol in presence of hydrochloric acid.

To determine the glomerular filtration rate (GFR) in man a large initial dose of inulin is injected, followed by a constant inulin infusion at a rate which compensates for its loss in the urine. A reasonably constant plasma level is thereby maintained. Inulin is non-toxic and physiologically inert. The inulin concentration in the plasma of venous blood (P), the urinary concentration (U) and the volume of urine excreted per minute (V) are determined.

$$GFR = CR_{in} = \frac{UV}{P}$$

The range of values in normal subjects of 1.73 sq m surface area are:

	Male		*Female*	
ERPF (ml per minute)	697	± 135.9	594	± 102.4
GFR (,, ,, ,,)	131	± 21.5	157	± 15.6
FF (,, ,, ,,)	0.19 ±	0.02	0.02 ±	0.03

Most kidney diseases are associated with low rates of ERPF and GFR and these determinations provide the most accurate method of assessing the extent of kidney damage. The FF is characteristically raised in idiopathic hypertension and cardiac failure and is low in acute nephritis.

Certain organic iodine compounds, e.g., iodopyracet (diodrast or 3:5-diiodo-4-pyridone-N-acetic acid), are completely or almost completely extracted from the blood during each passage through the kidney.

The relations of the tubules to the excretion of inulin, glucose, urea, creatinine and diodrast with clearance values for these substances are shown in Fig. 5.5.

125
125
125 × F

I
II
Y

$$\frac{UV}{P} = \quad 125 \qquad 0 \qquad 740$$

Inulin   Glucose   Deodrast

125
125

III
IV

70   175
Urea   Creatinine
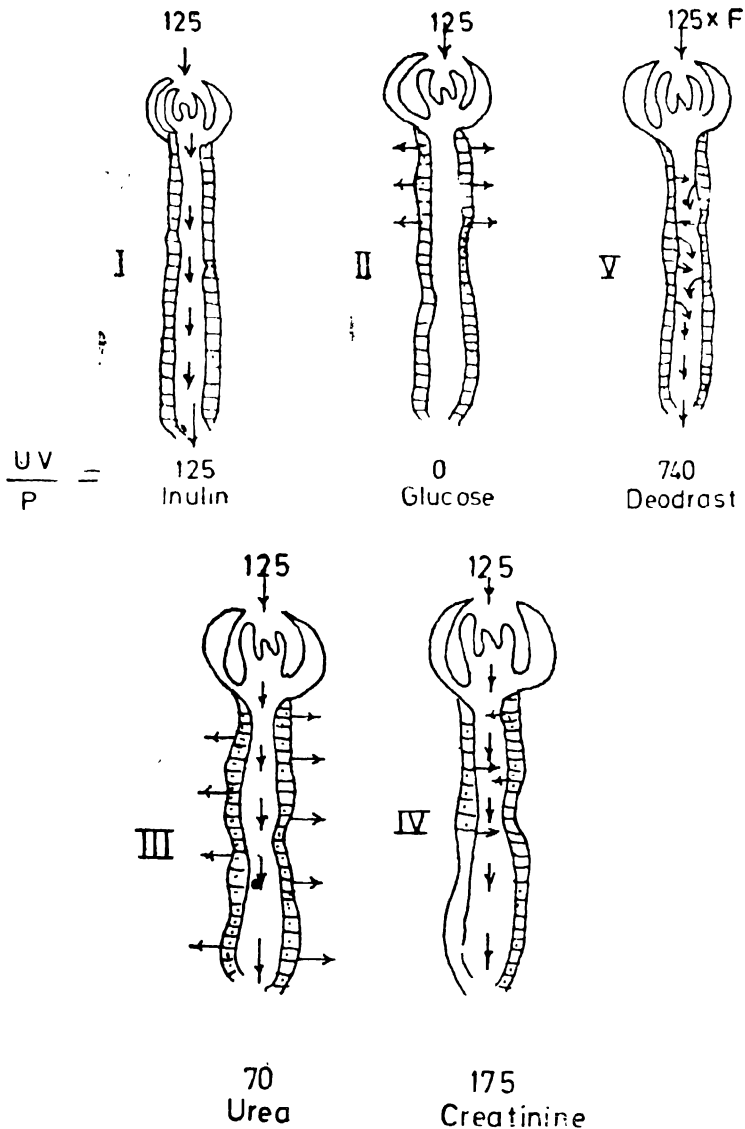
(From Gamble, Extracellular Fluid, 1954)

Fig. 5.1

I: Inulin is excreted solely by filtration with no tubular reabsorption.

II: Glucose, which is filtered at normal plasma level and rate of filtration, is completely reabsorbed by the tubule.

III: Urea, which is filtered, escapes in part from the tubular urine by diffusion.

IV: Creatinine, which is excreted by both filtration and tubular

excretion-like inulin, creatinine is filtered by the glomerulus and neither reabsorbed nor excreted by the tubules.

V: Diodrast, which is excreted by both filtration and tubular excretion.

UV/P: The clearance of each substance—the virtual volume of blood cleared per minute. F is the fraction of Diodrast filterable from the plasma.

Organic iodine compounds, such as Diodrast, often have unpleasant side-effects when injected and their use has been largely superseded by that of para-aminohippuric acid (PAH).

The clearance rate of PAH is 654 ± 163 in man and 592 ± 153 in woman both with surface area of 1.73 m². The test is performed by giving a large priming dose of PAH intravenously followed by continuous drip so as to maintain the plasma concentration compensating the loss in the urine. Plasma venous concentration is thus equivalent to the arterial concentration; the urinary concentration and volume—all are determined.

MAXIMAL TUBULAR REABSORPTION OF GLUCOSE Tm_G

The functional reabsorptive power of the tubular mass can be expressed quantitatively in terms of the ability of the tubule to reabsorb glucose. With plasma glucose concentration in normal amount, glucose is not excreted in the urine. When its concentration rises beyond normal level, progressively, the filtered load becomes too great for the tubular reabsorption powers and glycosuria occurs. The clearance of glucose (normally zero) becomes positive and rises linearly.

Tm_G in man with 1.73 m² surface area is 300–350 mg per minute. When the concentrations · of a substance in plasma and glomerular filtrate are equal, and the substance is not absorbed or excreted by the tubules, its clearance represents the rate of glomerular filtration. The glomerular filtration rate, as determined by clearance test, amounts to about 125 ml per minute for man of surface area of 1.73 m². The value is somewhat less for woman.

In normal man the glomerular filtration rate is remarkably constant over periods of years. Any substance which is not combined with plasma protein and shows lower clearance than inulin must be reabsorbed by the tubules. The clearance (C_m) normally ranges between 64 and 99 ml with an average of about 75 ml. This is 50 ml less than the glomerular filtration rate. This indicates tubular reabsorption of urea. About 40 per cent of the urea · present in glomerular filtrate is reabsorbed by the tubules. The average absorption of urea is indicated by:

$$\frac{\text{inulin clearance} (125) - \text{urea clearance} (75)}{\text{inulin clearance} (125)} = \frac{50}{125} = 0.4.$$

This means an average reabsorption of 40 per cent of urea. The clearances of many substances, such as Na^+, K^+, HCO_3^-, Cl^-, SO_4^{--}, HPO_4^{--} amino acids, glucose, uric acid, and ascorbic acid are less than that of inulin indicating their reabsorption by the tubules.

On the other hand there are substances which have higher rates of clearance than inulin indicating that these substances are secreted into the tubular fluid by the tubular cells. These substances are H^+, NH_4^+, phenolphthalein, N'-methylnicotinamide, penicillin, phenol red, p-aminohippurate, and iodopyracet (diodrast).

$$H_2N - \langle\text{benzene ring}\rangle - CO - NH - CH_2 COO:$$
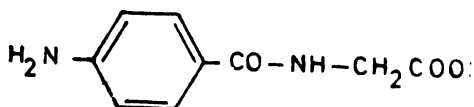
P aminohippuric acid

$$O = \langle\text{ring, 9, 9}\rangle N - CH_2COOH$$
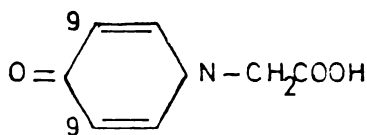
3,5- diiodo - 4 - pyridone -
N — acetic acid (Diodrast)

Phenol Sulphonphthalein Test (PSP)

Dyes are widely used for excretion-tests. Phenolsulphonphthalein (PSP), phenol red test is done by estimating the rate of excretion of the dye after the administration of the dye intramuscularly or intravenously. The Intravenous test yields better results as it eliminates the uncertainties of absorption. After the injection of the dye, urine specimens are called at 15,30,60 and 120 minutes—and the urine specimens are analysed to determine the dye-concentration. Presence of 25 per cent or more of the dye injected in the 15-minute urine indicates normal kidney function. Normally 40 to 60 per cent of the injected dye is excreted in the urine in the first hour and 20 to 25 per cent in the second hour. The dye excreted in the 15-minute specimen gives the most useful information. The dye is readily excreted by the tubules and the test does not give abnormal results until the impairment of renal function in extreme.

CONCENTRATION TEST

The ability of the kidney to produce a concentrated urine is a sensitive test of renal function. Impairment of the tubular function to perform osmotic work is an early feature of renal disease. When the kidneys do not work at all, the fluid excreted would have the same specific gravity as that of the glomerular filtrate, 1.010. And deviation from this specific gravity—either dilution or concentration—requires osmotic work by the

renal tubule. In severe renal damage the specific gravity of urine may be 1.010 to 1.012. A lesser degree of impairment may be the first detectable abnormality in renal function in chronic nephritis. Concentrating ability is normally diminished in old age, and may falsely appear to be affected in oedematous patients.

Addis Test

Fluids are withheld for 24 hours (from 8.00 A.M. on one day to 8.00 A.M. next day). The urine excreted upto 8.00 P.M. of the first day is discarded but that excreted from 8.00 P.M. of the first day to 8.00 A.M. of the next day is collected and its specific gravity determined. Normally the specific gravity of this specimen of urine exceeds 1.025 (upto 1.034). A specific gravity of less than 1.025 even after this period of water deprivation indicates renal damage where the concentrating power of the kidney is impaired. This may however occur during pregnancy, receding oedema or in diets inadequate in protein or salt.

The test is contra-indicated in cases of obviously impaired renal function or in hot weather or in diabetes with polyuria and adrenal insufficiency.

Mosenthal Test

Unlike Addis test, the fluid intake is not restricted. The bladder is emptied at 8.00 A.M. on the first day and the urine is discarded. Urine collections are made at two-hour intervals from 8.00 A.M to 8.00 P.M. and all the urine excreted during the 12-hour period from 8.00 P.M. to 8.00 A.M. is collected as one specimen. The specific gravity of each two-hour urine specimen and the volume and the specific gravity of the 12-hour urine specimen are noted. Normally the specific gravity of one or more of the two-hour specimens is 1.018 or more with a difference of not less than 0.009 between the highest and the lowest readings. The volume of the 12-hour night specimen is not less than 725 ml with a specific gravity of 1.018 or more. Mosenthal test is less rigorous than the Addis test.

Further Reading

R.F. Pitts, *Physiology of the Kidney and Body Fluids* (Chicago; 1969).

H.W. Smith, *The Kidney: Structure and Function in Health and Disease* (New York: 1951).

Bailey, *Textbook of Histology* (London: Williams and Wilkins, 1971).

B. Harrow, and A. Mazur, *Textbook of Biochemistry* (New York: Saunders 1958).

E.J. King, and I.D.P. Wootton, *Micro-Analysis in Medical Biochemistry* (London: Churchill, 1956).

H. Harper, *Review of Physiological Chemistry* (London: Lange, 1969).

E.S. West, *et al., Textbook of Biochemistry* (London: Collier-Macmillan, 1974).

Water and Mineral Metabolism

Introduction

Water is the most abundant of all compounds in the body. It is necessary in nutrition for the simple reason that about two-thirds of the body is composed of water. Water is present in every tissue with varying amounts: Saliva, 99.5 %, cerebrospinal fluid, 99%; embryonic brain, 91% brain (gray matter), 86%; kidney, 83%; thyroid, 82%; adrenals. 80%; blood, 79%; pancreas, 78%; muscle, 75%; liver. 70%; skin, 72%; tendon, 68%; cartilage, 67%; bones, 50 per cent; dentin, 10%. Its function is not just to add mass to living substance. Wastes of metabolic processes are excreted in a watery medium: hydrolytic processes f digestion take up a molecule of water with each enzyme action, sweating serves to eliminate wastes and to regulate the body temperature, and blood, the vehicle of transport of food substances, respiratory gases and cellular wastes, is largely water. A loss of 10 per cent of water content in man results in illness and deprivation of water brings about death much more rapidly than that of food—a loss of 20 per cent of water may cause death. Water is present in cellular and vascular spaces and small portions are also deposited in conjunction with protein and carbohydrate. The storage of fat is however, accompanied by little water. Life may continue for several weeks in spite of the loss of most of the body fat and 0 per cent of the tissue protein if water is given but no food.

More than 70 per cent is water in most of the tissues; even bone is nearly one-third water and adipose tissue (fat) contains quite appreciable quantity of water. Approximately half of the body water is present in muscle which accounts for about one-third of the body mass.

Minerals are important in nutrition. They are involved in an impressive variety of processes concerned with body function and structure. About fifteen mineral elements are essential to health as obtained from various salts in foods. Sodium and potassium constitute the major cations of

body tissues and chloride and bicarbonate are the important anions. Magnesium, phosphorus, calcium, iron, iodine, and others also play specific roles.

Chloride is especially important for it combines with sodium to form sodium chloride, the predominant compound in osmoregularity mechanisms of blood and cells. Chloride also combines with hydrogen to form hydrochloric acid which is necessary in gastric digestion. ATP synthesis needs phosphorus, so also does the tooth structure. Iron is required in haemoglobin and in the cytochromes of cellular respiration. Many other elements have known roles although they occur in trace quantities. As a matter of fact protoplasm contain almost all known elements but the importance of some of them has not yet been determined. It may well be that some have no function at all.

Water Metabolism

The cells of the body consist largely of water and they are embedded in a gel or matrix of protein and water. The water (fluid) of the body occurs, as it were, in two compartments or spaces; the water within the cells—the intracellular fluid—and the water outside them—the extracellular fluid. The part of the extracellular fluid circulating as plasma water in the blood vessels is called intravascular fluid which is separated by the capillary endothelium from the remainder is known as interstitial fluid.

The total body water is equal to 45 to 60 per cent of the body weight with an average of 55 per cent for adult men and 50 per cent for adult women. The body water has been assumed to be distributed throughout in two main compartments—the extracellular compartment which is sub-divided into plasma and interstitial fluid, and the intracellular compartment. The water within the cells is intracellular fluid and the water outside them is extracellular fluid. The intravascular fluid is that part of the extracellular fluid which circulates in the blood vessels as plasma water. This is separated from the remainder, the interstitial fluid, by the capillary endothelium. The distribution of body water in average normal man is indicated in Table 6.1.

TABLE 6.1

Source	Volume in litres	Percentage of body weight
Total body water (TBW)	42	60
Intracellular fluid (ICF)	26.5	38
Extracellular fluid (ECF)	15.5	22
Interstitial fluid	12	17
Intravascular fluid (Plasma)	3.5	5

From N. B. Talbot, R. H. Richie and J. D. Crawford, *Metabolic Homeostasis* (Cambridge, Mass.: Harvard University Press, 1959).

The body weight is made up of three components—fat, lean tissue and water. The lean tissue and water have their weights (masses) relatively constant in relation to the height of an individual and also in relation to one another. The fat mass is an independent variable which is unrelated to the other factors. About 60 per cent of the body mass in a lean adult male is made up of water. The water mass may fall to 40 per cent in a fat male of some height because the adipose tissue contains very little water. The absolute mass of water and the blood volume will, however, be quite similar in the two men. Thus the fat content determines the proportion of water in a tissue or in the whole body. About 14 per cent of the body weight is fat in men of average built and in women about 18 per cent. The weight of lean tissue (lean body mass) is the difference between the total body weight and that of fat. The proportion of water in lean body mass of man and animals is remarkably constant, which is about 73 per cent. The proportion of water in the body decreases progressively as age advances as indicated in Table 6.2.

TABLE 6.2

Age		Total body water as per cent body weight
Foetus	0–3 months	94.3
	3–6 ,,	89.1
	6–9 ,,	84.5
	up to 2 years	69.5
	2 to 7 years	6
	7 to 16 years	58.4
	22 to 58 years	51.7
	71 to 84 years	50.8

MEASUREMENT

The total amount of water in the body of an animal can be determined by drying the carcass completely and measuring the weight lost. •The water content of a piece of tissue or of a whole organ such as the liver, can in a similar way, be found directly.

In man the basic principle involves indirect methods in the measurement of a volume of a body fluid compartment. A substance is chosen which distributes itself evenly and completely throughout the space to be measured—throughout the plasma or the extracellular fluid. A known weight of he substance is injected into the compartment and time is allowed for mixing and a sample of fluid is withdrawn. The concentration of the substance in the sample is measured from which

the volume of the compartment can be calculated:

Example
    Amount of the substance injected   10 mg
    Concentration in the sample   0.5 mg/100 ml

    Volume of space $\dfrac{10}{0.5} \times 100 = 2000$ ml.

The substance injected must be non-toxic and capable of being estimated easily and accurately. The plasma volume, extracellular fluid (ECF) and the total body water (TBW) are commonly estimated. The difference between plasma and ECF gives the interstitial fluid (ICF) plus bone and connective tissue water. The intracellular water is the difference between TBW and ECF.

TOTAL BODY WATER

The distribution of heavy water, deuterium oxide (D_2O) and tritrated water (HTO) has been used in the living animal and human subjects as a method for the measurement of total body water. D_2O is estimated on the basis of its mass and HTO by its radioactivity. Both are treated by the body exactly as is ordinary water. After injection, about 2 hours are allowed for complete equilibration. A sample of blood is withdrawn and the concentration of D_2O or HTO is estimated. With the correction for the fat content, the total body water in various subjects is relatively constant and is expressed as percentage of the lean body mass (the sum of the fat free tissue). Reliable results are obtained by using the drug antipyrine which is distributed throughout the body water.

The total body water can also be calculated on the basis of specific gravity of the body. The body consists of fat, which is of relatively low density and fat-free tissue, which is of relatively high density. The subject is weighed in air and under water for measuring the specific gravity of the body. By this the proportion of the body which is fat tissue and that which is fat-free tissue can be calculated. The lean body mass can be estimated by this technique.

PLASMA VOLUME

The substance used must be rapidly and evenly distributed throughout the plasma, yet must not escape into the tissues or the urine. Two substances are commonly used. One is the Evan's blue dye (T-1824) which becomes firmly bound to the plasma albumin and is therefore treated as albumin. The other, human albumin tagged with radioactive iodine may be used (radio-iodine serum albumin—RISA). The dye or RISA is injected at zero time and small quantities of plasma samples are withdrawn at regular

intervals. The concentration in each sample is measured and plotted semi-logarithmically. The concentration falls sharply at first as thorough mixing takes place and then the rate of fall becomes steady. About 5 per cent of the remaining indicator is removed each hour. The normal plasma volume thus determined, ranges between 47 to 50 ml per kg body weight.

A different approach is to tag the red cells rather than the plasma. The haematocrit value gives the ratio of red cells to plasma in the blood, enabling the plasma volume to be calculated. The method involves the intravenous injection of radiophosphorus-labeled red cells (^{32}P) or radio-iodine-labeled human serum albumin. A known quantity of the tagged cells is injected and their dilution estimated after about 10 minutes from their concentration in an aliquot of blood or plasma.

EXTRACELLULAR FLUID

For the accurate determination of the extracellular fluid, the indicator used must diffuse into the most remote water droplets in bone and connective tissue and yet must not enter cells at all. No such substance exists. The matter is further complicated because a rather long time interval is required for complete mixing and many of the indicators are excreted or metabolized in significant amounts during this time. Some of the substances used are inulin, raffinose, sucrose, mannitol, thiosulphate, radiosulphate, thiocyanate, and radiosodium. These give values for ECF which progressively increase from about 16 per cent of lean body mass for inulin to about 30 per cent for radio sodium. Inulin, a relatively large molecule, is unlikely to penetrate all the recesses of the ECF and must give a value which is too low. Radiosodium, which undoubtedly penetrates cells to some extent, give a value which is too high. In cats, however, after nephrectomy to eliminate the complexities due to urinary excretion, mannitol, thiosulphate and radiosulphate all give the same value of about 23 per cent. This is probably the best estimate for ECF in a lean individual.

The volume of distribution of mannitol or inulin has been found to give a reasonable measurement of the volume of the interstitial and lymph fluid. The volume of the remaining components of the extracellular fluid have been estimated by direct chemical analysis of representative samples of the individual tissues. The interstitial fluid volume is equal to the extracellular fluid volume minus the plasma volume (Inulin space minus I^{131} albumin space).

INTRACELLULAR WATER

Intracellular water is equal to the total fluid volume minus the extracellular volume or it is represented by the D_2O space minus the inulin space. The interstitial fluid provides a fluid buffer between plasma and intracellular fluid. Plasma is subject to sudden variations in composition as a result of

absorption from the intestine, the interposition of interstitial fluid between plasma and intracellular fluid helps in the maintenance of the composition of intracellular fluid more constant than otherwise would have been possible. This arrangement permits the kidneys to compensate for the changes in plasma before they are reflected seriously upon the intracellular fluid.

Water Intake or the Availability of Water

The needs of the body for water are met in two ways: by direct intake, and by the oxidation of the food stuffs in the body. Water as such or water present in foods refers to direct intake. This is also called the performed water amounting to 1200 ml derived from liquids imbibed as such along with water in foods, about 1000 ml.

The amount of water liberated as a result of oxidation of food stuffs is about 10–14 g per 100 calories. This is called the water of oxidation or metabolic water, which amounts to about 300 ml. 100 g of fat when oxidized, produces 107 ml of water. The 100 g of fat is equivalent to 930 calories, which means that fat equivalent to 100 calories will produce 11.5 ml of water.

Oxidation of 100 g of carbohydrate yields 55 g of water and 100 g of protein, 41 g of water. Carbohydrate and protein equivalent to 100 calories each produces 13.5 and 10.1 ml of water respectively.

LOSSES OF WATER

The water leaves the body through four main channels. The water balance in the body is of great importance as the amount of water in the body tends to vary but little. Water is lost from the skin, as sensible and insensible perspiration; from the lungs, as water vapour in the expired air; from the kidneys, as urine; and from the intestines, in the faeces. The insensible losses cover the dermal loss (exclusive of visible perspiration) and the pulmonary loss.

Daily water losses and water allowances for normal individuals (not working or sweating) are indicated in Table 6.3.

DAILY TURNOVER OF WATER

The water in the body is being continually lost and replenished. A balance or equilibrium is maintained by the amounts of water entering and leaving the body daily. A positive balance in metabolic studies signifies that the intake is greater than the loss and the body is gaining fluid. A negative balance indicates that the total losses exceed the intake when the body is gradually losing fluid.

TABLE 6.3

Size	Losses			Allowances		
	Urine (ml)	Stool (ml)	Insensible (ml)	Total (ml)	ml/person	ml/kg
Infant (2–10 kg)	200–500	25–40	75–300 1.3 ml/kg/ hour	300–840	330–1000	165–100
Child (10–40 kg)	500–800	40–100	300–600	840–1500	1000–1800	100–45
Adolescent or Adult (60 kg)	800–1000	100	600–1000 0.5 ml/kg/ hour	1500–2100	1800–2500	45–30

From Butter and Talbot, *New England, J. Med.*, **231** (1944), p. 585.

Water balance is maintained in a healthy man in a temperate climate by drinking about 650 ml daily and in addition the water in his food and the water produced by the oxidative metabolism of carbohydrate, fat, and protein are available to him. Considerable amounts of water are also present in many of the foodstuffs he consumes and a normal diet may provide about 350 ml of water a day. Protein in his foodstuff provides relatively less water but the end-product of its metabolism, urea, requires water for its excretion in the urine. The water intake in health varies widely and is more than sufficient to compensate the losses in the urine and sweat or deficiencies in the water obtained from food.

A minimum amount of urine is required to be excreted daily so that the end-products of protein metabolism are eliminated by the kidneys. This volume of urine excreted (500 to 700 ml) is referred to as obligatory urine output. This obligatory urine output varies con siderably depending on protein catabolism and the electrolyte to be excreted. About 100 ml of water are lost daily in faeces. Water is continually lost through the skin by evaporation—amounting to about 500 ml a day. The loss of water and electrolytes on account of sweating in a hot environment may be considerable. Breathing is accompanied by water loss but not of electrolyte as the expired air is saturated with water vapour. The amount of water thus lost may be 300 ml or more per day. Exercise or fever raises breathing with the consequent increase in the loss of water.

The water lost through lungs, skin, and faeces amounts to about 900 ml per day and the urine accounts for about 700 ml a day. The total minimum loss from the body is about 1600 ml of water per day. This amount is about 4 per cent of the total water in the body. The minimum intake of water has to be therefore this amount minus the metabolic water. The water intake is however, much larger usually.

ADDITIONAL WATER LOSSES IN DISEASES

When the concentrating ability is impaired in kidney disease, the renal

loss of water may be twice the normal. Surgical operations, fever or physically debilitated conditions may cause a rise in the insensible losses of water. Extremely high extracellular water losses, as much as 2000 ml per day, may occur in high environmental temperatures. Diarrhoea or vomiting may lead to considerable loss of water from the intestine.

Thirst

The general bodily need for water produces the sensation of thirst. It does not result from a dry mouth merely. Thirst is produced from water deprivation long before the mouth becomes dry. Disease or absence of the salivary glands gives rise to the conditions known as xerostomia where there is no complaint of thirst. Secretion of saliva is stopped by an injection of atropine producing a dry mouth but still the person does not feel thirsty. Intravenous injection of hypertonic solutions, such as 5 per cent sodium chloride, produces thirst. Such solutions increase the osmotic pressure of the extracellular fluid and bring about a movement of fluid out of the cells, the osmolality of which is relatively increased. The thirst produced on alcohol consumption may have a similar explanation. The release of antidiuretic hormone from the posterior lobe of the pituitary gland is probably inhibited by alcohol. The consequent water diuresis leads to water depletion resulting in thirst.

FUNCTIONS OF WATER

Large proportion of the water in the body is of utmost physiological importance. It functions as solvent, as a carrier in transporting foods to tissues, and wastes from tissues, and as a regulator of body temperature. The electrolyte balance of the body is maintained with the help of water. A state of health is possible only so long as the osmotic pressure exerted by solutes remains in equilibrium.

Heat is gained by the oxidation of foodstuffs in the body. Heat is lost through urine and faeces to the extent of 1.8 per cent, warming of expired air by 3.5 per cent, vaporization from lungs by 7.2 per cent, evaporation from skin by 14.2 per cent, radiation and conduction from skin by 73.0 per cent.

FACTORS INFLUENCING THE DISTRIBUTION OF BODY WATER

Water can diffuse freely throughout the body. Gains or losses of water determine the amount of water in the different compartments roughly in proportion to their volumes. An increase or decrease in the volumes of water results in corresponding alterations in the concentrations of solute. A gain in body water is followed by a decrease in osmotic pressure, and a loss of body water gives rise to increased osmotic pressure. A convenient index of changes in osmolality is afforded by the estimation of Serum Na^+ concentration. An increase in serum Na^+ concentration above

normal of 135–145 meq per litre indicates hyperosmolality and a decrease hypoosmolality.

An increased concentration of solutes in the blood leads to increased secretion of the antidiuretic hormone causing increased reabsorption of water by the renal tubules. The limiting factor in this process is the osmotic gradient at the distal renal tubule. Thus osmotic forces are maintained by the solutes—the substances dis olved in the body water.

SOLUTES IN THE BODY

The solutes in the body fluids are important not only in directing fluid distribution in various compartments but also in the maintenance of acid-base balance. For a proper understanding of the mechanics of water retention and distribution, the solutes in body fluids may be conveniently divided into three categories as indicated below.

1. Organic compounds of small molecular size which includes glucose, urea, amino acids, etc. As these substances diffuse relatively freely across cell membranes, they are not important in the distribution of water in various compartments. If, however, they are present in large quantities, they aid in retention of water influencing thereby the total body water.

2. Organic substances of large molecular size, mainly the proteins, play an important role in the exchange of fluid between the circulating blood and the interstitial fluid. The protein fraction of the plasma and tissues exerts its influence mainly on the transfer of fluid from one compartment to another but not on the total body water.

3. Inorganic electrolytes are by far the most important, because of their presence in relatively large quantities in the body, both in the distribution and retention of body water.

The loss of body secretions from the alimentary tract is equivalent to a lose of fluid which is approximately isotonic with the extracellular fluid. Although the osmotic pressure of the body fluids is not altered by such a loss, the decline in volume of the extracellular fluid results in an increased secretion of the water-retaining antidiuretic hormone. The reduction of extracellular fluid volume induces, by a similar mechanism, the secretion of the salt retaining hormone aldosterone, which in turn increases the re-absorption of sodium and water by the renal tubules. The volume and osmolality of the extracellular fluid is controlled by the sensation of thirst, by the antidiuretic hormone and aldosterone and by other mechanisms not yet understood.

Various diseases can upset the normal balance of water and electrolytes. Both water and electrolytes are usually involved in such disturbances. In clinical practice states of combined salt and water loss (saline depletion)

and excess (saline overload) are commoner than those involving water alone.

Saline depletion is caused by loss of salt and water, commonly from the alimentary tract by vomiting or diarrhoea, less often in the urine. Occasionally the saline is lost in excess sweat or the exudate from severe burns. Rarely the diatary intake of sodium is insufficient. Saline depletion cause cramps, weakness and faintness, and clinical features of dehydration, diminished tissue turgor, hypotension and peripheral circulatory failure.

Primary disturbance of the function of the heart, kidneys or liver often results in saline overloading. Intravenous infusion of saline in excess occasionally causes saline overloading. Oedema and raised central venous pressure commonly result.

WATER DEPLETION

Inadequate water intake usually results in water depletion. This often happens in prolonged coma or in patients who are mentally confused when their need for fluids is not recognised. Rarely it is caused by abnormal loss, as when the kidney is unable to conserve water as a result of impaired production of vasopressin (diabetes insipidus) or in some forms of primary renal disease. No signs of dehydration are evident but often it is accompanied by fever and a raised concentration of plasma Na^+ (hypernatraemia). Conscious patients feel extremely thirsty and the urine is concentrated, except in diabetes insipidus.

Water overload or water introxication takes place when the water intake exceeds the ability of the kidneys to excrete it. This may occur in renal failure or after a surgical operation when the release of the anti-diuretic hormone is stimulated by the stress, and the intravenous infusions of solutions like 5 per cent glucose in water, which becomes hypotonic in the body as the solute is used up in metabolism. Water overload may occur in renal failure unless the intake is controlled. Water intoxication causes cerebral swelling giving rise to nausea and vomiting, headache, confusion, convulsions and coma. The Na^+ concentration in plasma is low (hyponatraemia).

'Sodium and potassium are the most important elements in the body fluids both from the standpoint of osmotic forces in directing the movement of water from one compartment to another and in controlling the total hydration of the body. Sodium is largely confined to the extracellular space and potassium to the intracellular space. Gamble has expressed sodium as the backbone of the extracellular fluid because of the fact that sodium, more than any other element, determines the quantity of extra-cellular fluid to be retained. It is for this reason the sodium intake is restricted in order to control overhydration in various pathological conditions.

POTASSIUM DEPLETION

Potassium resides mainly in the cells. A low plasma K+ is often associated with its deficiency but is not an accurate guide to the deficit in the whole body. Potassium leaves the cells under certain conditions such as found in prolonged gastrointestinal losses due to vomiting, diarrhoea, or prolonged gastric suction. Replacement of lost electrolytes with only sodium salts results in the migration of sodium into the cells to replace the potassium deficit. This causes profound alterations in cellular metabolism such as persistent alkalosis. Hypokalaemia causes tiredness, muscle weakness and polyuria. Prolonged administration of powerful diuretic drugs brings about this condition. An increased flow of urine is provoked by the diuretic drugs by inhibiting the activity of enzymes in the renal tubule that normally facilitate the absorption of Na+ and Cl−. These diuretics promote the exchange of Na+ for K+ in the distal tubule leading to increased excretion of K+ when the dietary intake of Na+ is low and the level of circulating aldosterone high. Hypokalaemia may also occur in chronic diarrhoea and repeated vomiting.

Electrolyte composition of extracellular and intracellular fluids in milli equivalents per litre are indicated in Table 6.4.

TABLE 6.4

Extracellular Fluid				Intracellular Fluid			
Cations		Anions		Cations		Anions	
Na+	145	Cl−	100	Na+	10	Cl−	10
K+	5	HCO₃−	27	K+	15C	HCO₃−	10
Ca++	2	PO₄−−−	2	Ca++	2	PO₄−−−	90
Mg++	2	SO₄−−	1	Mg++	15	SO₄−−	15
		Organic acids	5			Organic acids	
		Protein	19			Protein	52
Total	154		154		177		177

The plasma electrolyte composition is shown in Table 6.5.

TABLE 6.5

Cations	meq/litre	Anions	meq/litre
Na+	142	Cl−	103
K+	5	HCO₃−	27
Ca++	5	HPO₄−−	2
Mg++	3	SO₄−−	1
		Organic acids	6
		Proteins	16
Total	155		155

From Gramble, *Extra cellular fluid*, 1954.

The composition of interstitial fluid is similar to that of plasma, chloride replacing largely protein in the anion as shown in Table 6.6.

TABLE 6.6   INTERSTITIAL FLUID

Cations	meq/litre	Anions	meq/lttre
Na+	145	Cl⁻	120
K+	4	HCO₃⁻	27
Others	5	Others	7
Total	154		154

The intracellular fluid differs from that of plasma in that the potassium rather than sodium is the principal cation and, phosphate—largely due to the presence of phosphorylated organic compounds—rather than chloride is the principal anion. The chloride content of the intracellular fluid varies according to the metabolic circumstances. The amount of protein within the cell is also considerably larger than that in its 'extracellular environment.

It is now clear that sodium may replace potassium within the cell when sodium salts are administered to potassium-deficient subjects. The concentration of sodium and potassium within the cell is also influenced by the adrenocortical steroids and ACTH. The intracellular sodium may increase under the influence of these hormones.

Conversion of electrolyte concentrations to milliequivalents per litre is helpful in describing the chemical reactivity, particularly acid-base balance, where all reacting ions must be expressed in identical concentration units. Moreover when changes occur in the composition of the body fluid, compensatory shifts of one ion to make up for the losses of another take place. Thus the excessive losses of chloride over sodium in vomiting from the stomach result in a chloride deficit in the extracellular fluid⸱which is promptly compensated by an increase in bicarbonate to accompany sodium left uncovered by chloride loss. These changes can be readily understood and measured when all the reactants are expressed in same units—the meq per litre.

For conversion of electrolyte concentrations from mg per 100 ml to meq per litre, the concentration is first expressed in mg per litre which is then divided by the appropriate meq weight. The milliequivalent weight (meq) of an element is the millimolecular weight divided by the valence. The meq weights of some elements are given in Table 6.7

$$\left(\text{meq per litre} = \frac{\text{mg per 100 ml} \times 10 \times \text{valence}}{\text{atomic weight}} \right):$$

TABLE 6.7 MILLIEQUIVALENT WEIGHTS

Na	23	Cl	35.5
K	39	Cl as NaCl	58.5
Ca	20	HPO. as P	17.2
Mg	12	SO$_4$ as S	16

Examples

(1) Plasma sodium

$$= 322 \text{ mg}/100 \text{ ml}$$
$$= 322 \times 10 \text{ mg}/1000/\text{ml}$$
$$= 3220 \text{ mg/litre}$$
$$= \frac{3220}{22} = 140 \text{ meq/litre}$$

(2) Plasma chloride

(as NaCl)

$$= 603 \text{ mg}/100 \text{ ml}$$
$$= 603 \times 10 \text{ mg}/1000 \text{ ml}$$
$$= 6030 \text{ mg/litre}$$
$$\frac{6030}{58.5} = 103 \text{ meq/litre}$$

(3) Serum calcium

$$= 10 \text{ mg}/100 \text{ ml}$$
$$= 10 \times 10 \text{ mg}/1000 \text{ ml}$$
$$= 100 \text{ mg/litre}$$
$$\frac{100}{20} = 5 \text{ meq/litre}$$

(4) Plasma bicarbonate is measured by conversion to CO_2 and expressed in terms of volumes per cent (vol %). The CO_2 combining power, expressed as vol %, is divided by 2.3 to give its concentration in meq per litre. The conversion of the CO_2 combining power to meq of bicarbonate is based on the following.

One mol of a gas occupies 22.4 litres at 0°C. and 760 mm Hg and therefore 1 millimol (mM) of a gas occupies 22.4 ml which means 22.4 ml of gas is equivalent to 1 mM. Normal total blood CO_2 is 600 ml, which in terms of mM is 600/22.4 = 26.7 mM. 1 M of CO_2 is the same as 1 meq of CO_2.

The total CO_2 in blood includes carbonic acid, free CO_2, and bicarbonate. The bicarbonate fraction alone is calculated on an assumption that the carbonic acid and bicarbonate are present in a ratio 1:20. The

plasma bicarbonate fraction is derived by dividing the total CO_2 (as CO_2 combining power in Vol. %) by 2.3.

The Gibbs-Donnan equilibrium is of considerable importance in physiology and medicine. Intracellular fluid contains non-diffusible ions mainly in the form of organic acids and protein. The net charges on these ions at body pH is negative.

The volume and composition of blood plasma, main alimentary secretions, and sweat are indicated in Tables 6.8.

DEHYDRATION

Changes in water balance are almost always accompanied by changes in electrolytes. Dehydration may be caused by water loss or restriction in water intake. The rate of water loss exceeds the loss of electrolytes when the supply of water is restricted for any reason or when the losses of water are excessive. The extracellular fluid becomes concentrated and hypertonic to the cells. Water then shifts from the cells to the extracellular space to compensate giving rise to intracellular dehydration. Severe thirst, nausea, and vomiting, hot and dry body, a dry tongue, loss of coordination and a concentrated urine of small volume are associated with intracellular dehydration. This is corrected by giving water by mouth, or dextrose and water parenterally, until symptoms are alleviated and the urine volume is restored.

When an excess of water is ingested a relative deficit of electrolyte may occur. Administration of large quantities of electrolyte-free solutions may result in this condition of overhydration. Water and electrolytes are both lost more frequently and replacement with only water leads to a deficiency of electrolytes in presence of normal or excess total body water. The deficiency of sodium in the extracellular fluid causes hypotonicity of this fluid. Some water passes into the cells, which are hypertonic to the extracellular fluid, producing what is called intracellular oedema. The extracelluar fluid volume is diminshed. This is very damaging, resulting in diminished blood volume and the consequent fall in blood pressures, slowing of circulation, and impairment of renal function. The latter comfplication is serious as kidney is essential in restoring the normal equilibrium.

The patient becomes progressively weaker but does not complain of thirst and his urine volume also is not changed much. An elevated haematocrit, increased plasma total protein and a decreased sodium and chloride concentration in the plasma are characteristically associated with this type of dehydration.

Correction of Dehydration
Gastrointestinal secretions have a high content of electrolytes. Serious fluid and electrolyte deficits may occur as a result of loss of fluid from the gastro-intestinal tract unless prompt replacements are made to compensate

TABLE : 8

Fluid	Average Volume (ml/24 hours)	pH	Osmolality (m-Osmoles/kg)	Electrolyte concentrations (meq/l)			
				Na^+	K^+	Cl^-	HCO_3^-
Blood plasma	3670	7.4	—	135–150	3.6–5.5	100–105	24.6–28.8
Gastric juice	2500	7.6	285	31–90	4.3–12	52–124	0
Pancreatic juice	700	7.5–8.8	285	113–153	2.6–7.4	54–95	110
Bile	700–1000	7.4–7.8	285	134–156	3.9–6.3	83–110	38
Faeces	100	—	—	<10	<10	<15	<15
Mixed Saliva	1500	6.5	90–180	30	20	30	20
Sweat	500–4000	4.7–7.5	—	30–70	0–5	30–70	0

From O.M. Wrong, *Biochemical disorders in human di ase* (1970) and Randali S. Clin, *North America*, **32**: 3 (1952).

the losses. Withdrawl of fluid from the upper gastrointestinal tract may result in loss of chloride in excess of sodium.   This may occur in high intestinal obstruction, pyloric stenosis, gastric vomiting, or in continuous gastric suction.

Administration of sodium chloride   solution parenterally serves to repair the losses so that a proper adjustment of the electrolyte imbalance may occur.   A simultaneous replacement of potassium is also necessary. Fluid and electrolyte losses arising from the intestinal tract as in prolonged diarrhoea, pancreatic or biliary fistulas, etc., are characterized by the removal of a fluid high in sodium and bicarbonate content.   This leads to a relative chloride excess and a bicarbonate deficit.   This condition may be corrected by intravenous administration of a mixture of two-thirds isotonic saline solution and one-third sodium lactate solution.

Dehydration is often a complication not only in gastro-intestinal tract disturbances but also in diabetes mellitus, Addison's disease, uraemia, extensive burns, and shock.   The body weight remains more or less constant, with only slight variation, under conditions of proper nourish-ment and hydration.   Overhydration is indicated by rapid daily weight rise and a loss of 8 to 12 per cent body weight indicates a significant degree of dehydration, in case the loss of fluids is the cause for the loss of body weight.

Mineral Metabolism

Mineral elements constitute a relatively small amount of the total body tissues, nevertheless they play an essential role in many vital processes. Calcium, magnesium, sodium, potassium, phosphorus, sulphur, and chlorine are the mineral elements which the body needs in what might be called substantial amounts.   The body also needs smaller or trace amounts of iron copper, iodine, manganese, cobalt, zinc, and probably magnesium, molybdenum, and some others.

The general biological importance of calcium, phosphorus, iron, sodium, potassium, sulphur, and chlorine are known and some of their functions have been discovered.   Examples are: blood calcium and its role in neuromuscular irritability and in the clotting of blood, the effect of various irons on activation of enzymes which are involved in vital metabolic processes, and the activities of electrolytes in acid-base regulation.

Spectroscopic examination has revealed the presence of as many as 55 elements in plant or animal tissue, many of which occur only in traces. The trace elements are also known as 'oligo' elements, meaning scanty in Greek.   The concentration of the trace elements usually range between 1×10^{-6} and less than 1×10^{-12} g per gram of wet tissue.

More important of the elements and their quantitative occurrence as they are found in the human body, are listed below in Table 6.9.

TABLE 6.9 APPROXIMATE ELEMENTARY COMPOSITION OF THE BODY

Elements	Percentage
1. Oxygen	65
2. Carbon	18
3. Hydrogen	10
4. Nitrogen	3
5. Calcium	2
6. Phosphorus	1.1
7. Potassium	0.35
8. Sulphur	0.25
9. Sodium	0.15
10. Chlorine	0.15
11. Magnesium	0.05
12. Iron	0.004
13. Manganese	0.00013
14. Copper	0.00015
15. od...e	0.00004
16. Cobalt	— ⎫ Believed to be essential,
17. Zinc	— ⎬ quantitative data are
	⎭ not however, avaliable.

From Sherman and Lanford, *Essentials of Nutrition,* Macmillan.

In addition to common elements, milk has been ᴊund to contain aluminium, barium. boron, chromium, fluorine, lead, lithium, molybdenum, silver, zinc and others. Plant and animal tissues contain, in addition, cobalt, nickel, selenium, bromine, bismuth, arsenic, etc.

The functions of some of the mineral elements have been studied fairly well. Calcium and phosphorus are constituents of bone and teeth; important organic compounds found in the body, contain elements like iron, sulphur, and phosphorus. Sodium and chloride serve as electrolytes which are essential in the distribution of fluids in various body compartments and their retention as well as in the maintenance of acid-base balance; and it is possible that some like copper, play a role in catalyzing the enzyme action in the tissues.

Such elements are important not only by themselves in playing their specific roles but they are equally important in their relationship to one another, as the balance of ions in the tissues is often of importance. Normal ossification requires a proper ratio of calcium to phosphorus; the normal ratio between potassium and calcium must be maintained in the extracellular fluid to ensure normal activity of the muscle. When a frog's heart is immersed in solutions containing several salts at various

concentrations, it has been found that the heart continues to beat normally, provided the ratio K^+/Ca^{++} is that found in frog's blood. The irritability of tissues at various ionic concentrations appears to depend very largely upon the ratio:

$$\frac{Na^+ + K^+ + OH^-}{Ca^{++} + Mg^{++} + H^+}$$

The irritability increases when the concentration of the ions in the numerator is increased and the reverse is true when the ionic concentration in the denominator is increased. Ringer's solution is often used to retain the activities of tissues and tissue slices. It is made up of a solution of chlorides of potassium, sodium, calcium and magnesium in concentrations comparable to those in blood.

As mentioned earlier, sodium and potassium are the major factors in osmotic control of water metabolism. Other elements constitute an integral component of important physiological compounds. Examples are iodine in thyroxin, iron in haemoglobin, zinc in insulin, cobalt in vitamin B_{12}, sulphur in thiamine, biotin, coenzyme A, and lipoic acid.

Seven principal mineral elements are needed by the animal body. They are calcium, magnesium, sodium, potassium, phosphorus, sulphur, and chlorine. These elements constitute 60 to 80 per cent of all the inorganic material in the body. At least seven other minerals are required in trace amounts. They are iron, copper, iodine, manganese, cobalt, zinc, and molybdenum. Several others are found in the tissues but their functions are not clear. They are fluorine, aluminium, boron, selenium, cadmium and chromium.

The metabolism of food minerals does not involve radical changes of molecular form which occur in the metabolism of protein, carbohydrate and lipids. Calcium (Ca^{++}), magnesium (Mg^{++}), potassium (K^+), and sodium (Na^+) taken in food as salts of organic or inorganic acids or associated with proteins or lipids, constitute the positive mineral ions and are associated with the appropriate negative ions in the body after absorption. Tricalcium phosphate may undergo change to more soluble secondary phosphate before absorption and then circulate partly in this form in the body after absorption. The calcium ion may become partly associated with plasma protein, protoplasmic protein, or organic or other inorganic acids. The phosphate radical may get converted into an organic ester in the blood or tissue cells. The positive and negative mineral ions, not required as body structural units, do not generally undergo much chemical alterations than an exchange of partners during metabolism and excretion.

Calcium

FUNCTIONS

This mineral element occurs in greater abundance in the body than any

other. About 1200 g of calcium are present in an adult weighing 70 kg. The normal level of blood calcium in humans and in many other animals ranges between 9 to 11 mg per 100 ml of serum. The cells contain negligible amounts. The relationship $[Ca] \times [P] = 36$ holds fairly well. (Ca) and (P) are expressed in mg per 100 ml. About 60 per cent of the calcium in the blood is in a diffusible form and the remainder is quite nondiffusible, probably attached to the erum albumin. The diffusible fraction is referred to as ultrafiltrable or the ionized blood calcium. The ionic calcium constitutes the physiologically active fraction.

Calcium is the chief constituent of bone, which accounts for 99 per cent of the total amount of calcium in the body. The element may occur as a double salt of the carbonate and phosphate, $CaCO_3 \cdot nCa_3(PO_4)_2$, n being not less than 2 nor greater than 3. The principal inorganic salt in bone appears to be a hydroxyapatite or a hydrated tricalcium phosphate. Smaller amounts of calcium are found in teeth (36 per cent in enamel), skin, and blood.

The ionized calcium is of great importance in blood coagulation, in the function of the heart, muscles and nerves, and in the permeability of membranes. A deficiency of the parathyroid hormone brings down the concentration of ionic calcium below the normal level, which affects the central nervous system and produces an increased irritability of the peripheral nerves At a later stage, muscle spasms, affecting the face, hands, and feet and general convulsions take place. A decrease in the ionized fraction of calcium causes tetany. This may be due to an increase in the pH of the blood producing alkalotic tetany or gastric tetany or due to lack of calcium because of its poor absorption from the intestine, decreased dietary intake, increased renal excretion as in nephritis or parathyroid deficiency.

SOURCES

Milk and cheese are particularly rich sources, milk containing about 1.4 g of calcium per litre and cheese, 5-10 g per kg. Other dietary sources include egg yolk, beans, lentils, nuts, figs, cabbage and cauliflower. The mere fact that a food is rich in calcium or in any other element, does not necessarily mean that such diet will bring about 100 per cent absorption and assimilation. Various studies have shown that 20 to 30 per cent of calcium in milk is utilized by the human organism. Oxalic acid, citrates and phytic acid present in green vegetables and other foods, interfere with the absorption of calcium owing to the insolubility of their salts resulting in the poor utilization of the element. Present-day consumption of large quantities of refined cereals, with much of the original calcium in the whole grain lost, and sugar, which is devoid of minerals, makes the problem of providing minerals needs difficult.

REQUIREMENTS

The daily need for calcium by adult is not less than 0.7 g. 0.8 to 1.0 g represents an optional rather than a minimal allowance. During second and third trimesters of pregnancy and during lactation the daily requirement is 1.2 to 1.3 g. Infants under one year need 400 to 600 mg daily. Children between 1 to 18 years need 0.7 to 1.4 g daily.

Additional calcium may be administered in the form of its salts, such as carbonate, lactate, gluconate as well as dicalcium phosphate. Hypercalcaemia and possibly widespread excessive calcification may result from a high intake of calcium in the presence of a high intake of vitamin D such as may occur in children.

ABSORPTION

The ability of different individuals to utilize calcium in foods varies considerably. 15 per cent of the dietary calcium is absorbed on a high protein diet, on a low-protein diet the calcium absorption may be 5 per cent. Phytic acid in cereal grains and oxalates in foods interfere with the absorption of calcium by forming insoluble calcium salts in the intestine. Other intestinal factors which influence calcium absorption are the following:

pH Value
The absorption of calcium is increased by increasing the acidity of the intestinal contents and more calcium appears to be absorbed from concentrated than from dilute solutions. An increase in acidophilic flora (lactobacilli lowers the pH) favours calcium absorption.

Phosphate
The ratio of calcium to phosphorus in the diet has an important bearing on the degree of absorption, and thus on blood levels of both the elements. The faecal excretion of both increases with an excess intake of either in the diet. A ratio of Ca:P in the diet within the limits 1:2 to 2:1 allows for the optimum utilization of both elements. Absorption is decreased when this ratio is outside the limits. When the Ca:P ratio is high, much, $Ca_3(PO_4)_2$ will be formed and the calcium absorption will be diminished.

Free Fatty Acids
Under normal conditions, intake of more or less fat does not affect much the absorption of calcium from the intestine. But this is not so when the fat absorption is impaired. Under such condition much of the free fatty acids which is not absorbed, reacts with the free calcium to form insoluble calcium soaps with a marked loss of food calcium in the faeces. The loss of unhydrolysed fat in the faeces does not bring about calcium loss to the body. Calcium loss may be marked in diarrhoea and steatorrhoea.

Vitamin D

There is a deficiency of phosphorus in the common form of rickets—calcium may also be involved. Vitamin D, possibly by regulating the utilization of calcium from the intestine, influences the extent to which the body uses the element.

DISTRIBUTION

About 99 per cent of the total amount of calcium in the body, as stated before, occurs in the bones and teeth. Its distribution in body fluids or tissues is shown in Table 6.10.

TABLE 6.10

Fluid or Tissue	mg/100 ml or 100 g	meq/l
Serum	9 –11	5
CSF	4.5–5	2
Muscle	70	
Nerve	15	

METABOLISM

Very little calcium is present in the blood cells. Most of the blood calcium therefore occurs in plasma or serum, where it exists in three fractions: ionized (60 per cent) or diffusible calcium, protein-bound calcium (non-diffusible) and a small amount as citrate. All the three forms of calcium in the serum are in equilibrium with one another. All the three are measured in the usual determination of serum calcium. The protein-bound (nondiffusible) and the free (ionized, diffusible) calcium can be separated using only 5 ml of serum by ultracentrifugal method. The normal range of free calcium in the serum has been found to be 49.7 to 57.8 per cent of the total calcium (mean, 53.1 ± 2.6 per cent) at pH 7.35 and a temperature of 37°C.

Tetany is caused by a decrease in the ionized fraction of serum calcium. Serum calcium levels are low when the retention of phosphorus is increased, as in renal tubular disease.

Normal ossification depends on a proper ratio of Ca:P in the serum. In children the product of serum calcium and phosphorus in mg per 100 ml is normally about 50. This may be below 30 in rickets.

Unlike sodium and potassium, much of the calcium is excreted by the bowel—about 65 to 75 per cent of the element is found in faeces and 25 to 35 per cent in the urine. Renal glomeruli filters about 10 g of calcium in 24 hours, but only 200 mg approximately is excreted in the urine. The maximal renal tubular reabsorption for calcium (Tm_{Ca}) is about

4.99±0.21 mg per minute. Excretion in faeces accounts for most of the calcium eliminated from the body; this is almost entirely the unabsorbed dietary calcium. The amount of calcium reexcreted into the intestine is very small.

Parathyroid Activity and Calcium Metabolism
Parathyroids profoundly influence the metabolism of calcium. Hyperactive, hyperplastic, or adenomatous parathyroid glands cause hyperparathyroidism giving rise to an excess of calcium in the blood. Hypercalcaemia occurs with serum calcium rising to 12 to 22 mg per 100 ml accompanied by a decrease in serum phosphate, decreased renal tubular reabsorption of phosphate, increased phosphatase activity, rise in urinary calcium and phosphorus from bone decalcification, dehydration and haemoconcentration. Increased renal losses of phosphorus causes a decrease in serum phosphate which in turn elicits an increase in serum calcium in order to maintain the Ca:P product. The extra calcium and phosphorus is lost from the soft tissues and from bone by increased osteoclastic (bone-destroying) activity. At the same time, hardening of various organs—heart, lungs, arteries, etc., occurs due to calcium deposition. The excess calcium is derived from the bones, which, in turn become soft and weak.

The total serum calcium may not rise significantly for the diagnosis of hyperparathyroidism with certainty. In hyperparathyroidism due to parathyroid adenoma, the ionized calcium level has been found to rise from the normal range 5.9 to 6.5 mg per 100 ml to 6.1 to 9.5 mg per 100 ml of serum, the protein-bound serum calcium level remaining normal or slightly above normal in majority of the cases. Determination of the ionized fraction of serum calcium appears to be of better diagnostic value in hyperparathyroidism particularly when the serum calcium is within the normal range.

Hypoparathyroidism
The concentration of serum calcium level may fall to 7 mg per 100 ml after the surgical removal of diseased parathyroid glands. This drop in serum calcium level elicits an increase in serum phosphate and a decrease in urinary phosphates. The urinary calcium is also extremely low.

Rickets
Faulty calcification of bones may occur due to low Vitamin D content of the body, a deficiency of dietary calcium and phosphorus or due to a combination of both, with the result rickets develops. The serum phosphate concentration in rickets is usually low or normal, except in renal disease where it may be increased. The serum calcium is either normal or decreased. Poor absorption of calcium and phosphate results in increased excretion of these elements in the faeces and a decreased urinary

calcium and phosphate. In rickets the serum alkaline phosphatase is characteristically raised.

Renal Rickets
Renal tubular defect which is usually inherited, interferes with the reabsorption of phosphorus giving rise to renal rickets. Administration of Vitamin D in ordinary dosages cannot relieve renal rickets.

Renal Disease
The serum calcium level may decrease in severe renal disease, partly due to increased losses in urine but mainly due to increased serum phosphorus which causes a compensatory decrease in serum calcium. Calcium and phosphorus are essentially concerned in the formation of bone and teeth but the mechanism underlying the process of calcification is not clear. Growth is intimately connected with bone development and so with calcium.

Apart from the importance of calcium in the structure of bone and teeth and in its influence upon the excitability of the motor system, Ca^{++} ion plays a role in blood clotting. Calcium is necessary for the activation of a number of enzymes, such as lipase, succinic dehydrogenase, adenosine triphosphatase, and certain proteolytic enzymes. It is not known whether such an activation is essential or even of importance to the normal metabolic processes.

The Ca^{++} ion is directly related to muscle contraction. The ability of all types of muscles to contract is lost in the absence of calcium. The ratios of calcium to other cations is of course, of importance in muscle contraction. Calcium is also functionally involved in the transmission of nerve impulses. Calcium generally decreases the membrane permeability to balance the opposite action of sodium and potassium.

The physicochemical action of calcium in the regulation of water balance is insignificant because of its presence in small amounts in body fluids as compared to other regulatory ions like Na^+, K^+, Cl^-, and HCO_3^-.

Phosphorus

The element phosphorus is present in every cell of the body, but most of it, about 80 per cent of the total, occurs combined with calcium in the bones and teeth. About 10 per cent of phosphorus is in combination with proteins, lipids, and carbohydrates and in other compounds in blood and muscle. The remaining 10 per cent is distributed widely in various chemical compounds. The latter include phosphatides, nucleic acid, phosphoprotein (as casein), adenylic acid, coenzyme, yellow enzymes, thiamine phosphate, phosphocreatine, hexosephosphates and triosephosphates.

The total phosphorus content of the body is estimated to be about 700 g of which 600 g is present in the skeleton, 57 g in muscle, 5 g in brain, 2 g in blood.

REQUIREMENTS AND SOURCES

The daily needs of phosphorus have been calculated to be about 1.32 g. A ratio of Ca:P of 1:1 is the recommended dietary intake except in infancy when the ratio recommended is 2:1 based on the ratio found in human milk. The foods particularly rich in phosphorus are cheese, nuts, eggs, meat, and milk. An adequate intake of calcium generally provides the adequate intake of phosphorus also as their distribution in foods is very similar. Cow's milk contains more phosphorus than calcium. Foods rich in calcium and protein are excellent sources of phosphorus.

FUNCTIONS

The functions of phosphorus are numerous. They involve the chemistry of the blood, acid-base balance of the body, skeletal growth, tooth development, muscle metabolism, intermediary metabolism of carbo-hydrates, fat, protein, and brain, the activity of enzymes and so on. The phosphate occupies an important place in the high-energy bonds, such as ATP on intermediary metabolism. Synthesis of phospholipids, constituents of cell membranes, nervous tissues require phosphates. All through the body phosphates contribute significantly to buffer systems. The phosphate containing compounds RNA and DNA are important in protein synthesis and genetics. A number of coenzymes are phosphate compounds.

DISTRIBUTION

The distribution of phosphorus in the body fluids or tissues is indicated in Table 6.11.

TABLE 6.11

Fluid or tissue	mg/100 ml or 100 g	mM/l
Blood	40	
Serum (inorganic)		
Children	4-7	1.3-2.3
Adults	3-4.5	0.9-1.5
Muscle	170-250	
Nerve	360	
Bones and teeth	22,000	

METABOLISM

The metabolism of calcium and phosphorus is interlinked. The dietary Ca:P ratio influences the absorption and excretion of both the elements. Ordinary diets provide about one and a half time as much phosphorus as calcium. Considerable phosphorus is obtained from proteins and an adequate intake of protein and calcium from foods ensures adequate phosphorus requirement. Milk with 93 mg per cent and cheese with 200-900 mg per cent of phosphorus and meat and fish products with 100 to 200 mg per cent of phosphorus are good dietary sources.

About 60 to 80 per cent of the ingested phosphyrus is excreted by the kidney and the remainder by the bowel in health. In the case of calcium, the faeces accounts for 65 to 80 per cent and the remainder in urine. The amounts of calcium and phosphorus excreted by the two pathways may be reversed or practically all of one element may be eliminated through one of the pathways under various conditions of altered calcium-phosphorus metabolism or under specific dietary conditions. Excess intake of either element causes an increased excretion of the other. The optimal ratio of dietary Ca:P is 1:1 when vitamin D intake is adequate.

A temporary decrease in serum phosphate occurs with an increase in carbohydrate metabolism, such as during the absorption of carbohydrate. Absorption of some fats also causes a similar decrease in serum phosphate. A lower concentration of organic phosphorus and a higher inorganic phosphorus in the serum are observed in diabetes mellitus. Serum phosphate may be as low as 1 to 2 mg per 100 ml in rickets of the common low-phosphate variety.

Acidosis in severe renal disease is primarily due to phosphate retention. The serum phosphate under this condition is increased with the resultant decrease in serum calcium level.

Hypoparathyroidism brings about an elevated blood phosphorus. Growing children have higher blood phosphorus concentration as it occurs in acromegaly indicating a relationship between phosphorus metabolism and growth hormone. In hyperparathyroidism, sprue and in celiac disease the blood phosphorus levels are low. Renal tubular defect in the reabsorption of phosphate causes characteristic low blood phosphate and an elevated serum alkaline phosphatase. Vitamin D-resistant rickets, Milkman's syndrome (idiopathic osteomalacia in adults) and the De ,Toni-Fanconi Syndrome (hypophosphataemic-glycosuric rickets) belong to this category of renal tubular defect.

•The use of isotopes has shown that the body constituents, both organic and inorganic, are in dynamic equilibrium with each other and with ingested foods In bones and teeth also the constituents are in a dynamic state, undergoing continuous degradation and synthesis.

Radioactive tracers have been used in investigating metabolic problems which indicates that appreciable amount of the phosphorus from food move rapidly to bones, teeth, muscle, etc. It has also been shown that

lecithin could be isolated from the brain of rats, mice and rabbits, which contained the radiophosphorus (^{32}P). Ingestion of sodium phosphate containing radiophosphorus, ^{32}P in goats results in deposition of labeled phosphorus in milk within 3 to 4 hours. The isotope ^{32}P is found in the casein of the milk also. This indicates that the phosphorus used in the synthesis of casein in the mammary gland must have been derived from the inorganic phosphate in blood, which, in turn, depends on the inorganic phosphate of the food.

Magnesium

The element is an essential constituent of the chlorophyll molecule, and therefore is of importance to plant life. It is also of importance to animal life. Its deficiency in the diet of rats causes vasodilatation and hyper-irritability of the nervous system which resembles in some ways, the tetany due to calcium deficiency. The tetany caused by the deficiency of magnesium is different from that brought about by calcium deficiency.

Function and Distribution

About 21 g of magnesium is present in the human body.

About 70 per cent of the total magnesium found in the body is located in the bones combined with calcium and phosphorus as complex salts. The remainder is in the soft tissues and body fluids. Magnesium is one of the principal cations of soft tissues. 1 to 3 mg of magnesium per 100 ml or 1.9 meq per litre is present in blood. The serum contains less than half as much magnesium as the cells. This is in contrast to calcium, which is found almost exclusively in serum. Cerebrospinal fluid contains about 3 mg per 100 ml or 2.40 meq per litre.

The total quantity of magnesium in the body is far less than that of calcium but more of the former is found in muscle. About 21 mg of magnesium per 100 g is present in muscle tissue, as compared to 7 mg of calcium. The magnesium functions probably in conjunction with the adenylic acid as a coenzyme in carbohydrate metabolism and also in yeast fermentation. Many enzymes require Mg^{++} in addition to ATP for their activity.

Rich sources of magnesium include derivatives of cocoa, various nuts, soyabeans and some sea foods containing about 100 to 400 mg per 100 g. Whole grains and raw dried beans and peas may contain 100 to 200 mg of magnesium per 100 g.

The approximate amounts of calcium, phosphorus and magnesium in grams as they occur in 100 g of different foods, are indicated in Table 6.12.

TABLE 6.12

Food	Calcium	Phosphorus	Magnesium
1. Beef (lean)	0.007	0.218	0.024
2. Eggs	0.067	0.180	0.011
3. Eggyolk	0.137	0.524	0.016
4. Milk	0.210	0.093	0.012
5. Cheese	0.931	0.680	0.037
6. Wheat	0.045	0.423	0.133
7. Potatoes	0.014	0.058	0.028
8. Corn meal	0.018	0.190	0.084
9. Oranges	0.045	0.021	0.012
10. Almonds	0.239	0.465	0.251
11. Spinach	0.067	0.068	0.037
12. Beans (dried)	0.160	0.470	0.156
13. Linseed meal	0.413	0.741	0.432
14. Cotton seed meal	0.265	1.193	0.462

From Schmidt and Greenberg, *Physiol. Rev.*, **15**; p. 300.

REQUIREMENTS

The wide spread occurrence of magnesium in both plant and animal tissues ensures an adequate intake except under extreme conditions. Tetany is produced when the blood levels of magnesium is low & it happens with low levels of calcium. The serum magnesium level as low as 0.7 to 1.6 mg per 100 ml or 0.6 to 1.33• meq per litre causes muscle hyperirritability (muscle spasm or convulsions) indicating marked hyperactivity to auditory or mechanical stimulation. The parenteral administration of magnesium sulphate promptly reverses the clinical and chemical changes associated with the illness. Excess plasma magnesium decreases muscle and nerve irritability and levels of plasma magnesium as high as 20 mg per 100 ml induce anaesthesia.

The recommended dietary intake of magnesium is 350 mg per day for adult men and 300 mg per day for adult women. Several dietary constituents have been found to interfere with retention or increase the requirement of magnesium. They are calcium, protein, and vitamin D. It is claimed that alcohol increases the magnesium loss from the body. The daily magnesium intake may be as high as 7 to 10 mg per kg body weight per day with the western diet because of its high calcium, protein, and vitamin D content and because of the consumption of alcohol more commonly prevalent among the western people. Sub-acute or chronic deficiency of magnesium is not readily detectable.

METABOLISM

The metabolism of magnesium is similar to that of calcium and phosphorus. It is not safe to assume that factors governing the absorption and excretion of calcium are the same as those that govern the metabolism of magnesium. The parathyroid hormone is important in regulating calcium blood levels and excretion, but it has little effect on magnesium in these respects. On the other hand, magnesium, like calcium, absorption from the intestine is markedly decreased on a diet containing excess phytic acid.

Use is made of isotopic ^{28}Mg in measuring the absorption and excretion of magnesium from the intestine in human subjects.* About 44.3 per cent of the ingested radioisotope ^{28}Mg is reported to be absorbed on an average diet containing 20 meq of Mg per day. On a low mg diet, 1.9 meq per day, absorption may be about 75.8 per cent which may be only 23.7 per cent on a high Mg intake (47 meq per day). The rate and duration of absorption of ingested magnesium indicate that most of it occurred from the small intestine and little or none from the colon. Absorption of magnesium from the intestine does not appear to be related to the magnesium stores in the body. About 10 per cent of the amount absorbed is excreted in the urine, in the first 48 hours of radio-active magnesium administration, indicating excellent renal conservation of body magnesium. The average urinary magnesium content is only about 6 to 20 meq per litre. Aldosterone increases the renal clearance of magnesium as it does also the excretion of potassium.

An antagonism between calcium and magnesium appears to exist. Normal serum magnesium concentration is 2.4 mg per 100 ml. When the magnesium level is raised to 20 mg per 100 ml of serum by intravenous injection of sufficient amount of magnesium, immediate and profound anaesthesia alongwith paralysis of voluntary muscles results. Immediate reversal of the effect takes place by the intravenous injection of a corresponding amount of calcium. Thus these two cations appear to exert differing effects on cell permeability. The magnesium content in the cells is about ten times that in the extracellular fluid. Normally the magnesium level in blood plasma is 2.4 mg per 100 ml and in the muscle cells, it is about 23 mg per 100 g. This difference in distribution is not found with calcium as it is so particularly prominent with sodium, potassium, and magnesium. Magnesium and potassium are normally concentrated within the cell and sodium outside. Profound physiological changes occur by an alteration of this pattern.

Rats on low magnesium diet, 0.18 mg per 100 g of food, develop vasodilatation and hyperaemia, hyperirritability, cardiac arrhythmia and convulsions ending in death. Tetany is probably due to low magnesium level in serum when the dietary intake of magnesium is low. The serum calcium levels under such condrtion, remain within normal range.

*L.A., Graham, J.J. Caesar and A.S.V. Burgen, *Metabolism*, **9**, (1960), p. 646.

Magnesium deficiency in man causes a clinical syndrome, characterized by muscle tremor, twitching, occasionally convulsions and often by delirium. Magnesium deficiency has been observed in large group of patients with chronic alcoholism and in a few post operative patients as well as in those with pyloric obstruction and hypochloremic alkalosis. The serum magnesium levels in all these cases are moderately reduced. In clinical hyperthyroidism a decrease in serum magnesium has been observed.

The serum magnesium level may not correlate well with the intracellular concentration of the ion. The magnesium content of the red blood cells has been measured. The mean erythrocyte Mg in healthy adults has been found to be 5.29 ± 0.34 meq per litre, with the mean plasma level as 1.80 ± 0.13 meq per litre. In patients with the magnesium deficiency symptoms, the erythrocyte magnesium level has been reported to be 3.9 ± 0.75 meq per litre, plasma concentration being 1.5 ± 0.28 meq per litre. The levels of magnesium in the erythrocytes and plasma rise to normal by the administration of magnesium sulphate intramuscularly.

Magnesium level in serum increases in renal failure. The mean erythrocyte and plasma levels of magnesium in uraemia and associated depression of the central nervous system, have been found to be 8.84 ± 1.71 meq per litre and 3.17 ± 1.30 meq per litre respectively. Elevated serum or plasma levels of magnesium are reliable evidence of total magnesium excess in the body. A deficit of magnesium in the body may not become apparent by measuring the serum magnessium level, in such cases the erythrocyte magnesium levels are more informative.

Sodium

Sodium is the principal cation component of the extracellular fluid. It is associated with chloride and bicarbonate. It has two important functions— in the acid-base balance of the body and in large measure, for the total osmotic pressure of the extracellular fluids and thus in the protection of the body against excessive fluid loss. Sodium also functions in the preservation of normal irritability of muscle and the permeability of the cells.   -

REQUIREMENTS AND SOURCES

The main source of the element is its salt, sodium chloride, employed in cooking and seasoning and some is derived from the foods that are taken. The ordinary daily diet contains about 10 to 15 g of sodium chloride.

Sodium is very easily absorbed about 90 to 95 per cent appearing in the urine. The requirements of sodium have been established from observations on urinary losses. Addition of excess of sodium to the diet causes an excessive excretion of potassium, and vice versa. This

perhaps explains as to why herbivorous animals so often have a craving for salt (sodium chloride). Their food is particularly rich in potassium causing an excessive excretion of sodium. Foods of vegetable origin are richer in potassium than in sodium.

Adults maintained on daily intakes of only 100 to 150 mg., sodium lost is less than 25 mg per day. This amount is perhaps the minimum losses in the sweat. The normal obligatory daily losses of sodium have been estimated to be 5 to 35 mg in urine; 10 to 125 mg in stool; 25 mg through skin (not sweating) with a total of 40 to 185 mg.

Sweat constitutes the most variable source of salt loss but this loss can be minimized under conditions of prolonged exposure to high temperatures by allowing a few days for adaptation.

Intake of a maximum of 5 g of sodium chloride per day has been recommended for adults without a family history of hypertension. This intake is ten times the amount at which adequate sodium chloride balance can be maintained. A daily intake of about 1 g of sodium chloride is recommended for persons with a family history of hypertension.

DISTRIBUTION

The inorganic portion of the skeleton contains about one-third of the sodium content of the body. Most of the sodium is present in the extracellular fluids of the body. The distribution of sodium in body fluids or tissues is indicated in Table 6.13.

TABLE 6.13

Fluid or Tissue	mg per 100 ml or per 100 g	meq per litre
Whole blood	160	70
Plasma	330	143
Cells	85	37
Muscle tissue	60–160	
Nerve tissue	312	

The approximate concentration of cations in blood and muscle is shown in Table 6.14.

TABLE 6.14

Cation	Serum meq per litre	Red cells meq per litre	Muscle meq per kg
Na+	143	0	31
K+	5	108	93
Ca++	5	0	4
Mg++	3	2	19

METABOLISM

A relationship between the adrenals and the metobolism of sodium has been established. The adrenocortical steroids influence the metabolism of sodium. The removal of the adrenals or adrenocortical insufficiency leads to a decrease in serum sodium and ˜n increase in sodium excretion from the body.

Sodium depletion occurs in chronic renal disease particularly when it is accompanied by acidosis. This happens as a result of poor tubular reabsorption of sodium and due to loss of sodium in the buffering of acids.

Extreme sweating due to high environmental temperature or exertion may cause a considerable loss of sodium chloride from the body, as to develop muscular cramps of the extremities and abdomen, headache, nausea, and diarrhoea.

The serum sodium levels may not reflect accurately the total body sodium. A low serum sodium concentration (hyponatremia) may occur by ingestion of a large quantity of saltfree fluids. This does not indicate body sodium depletion actually; it is rather an effect of overhydration. A low serum sodium may similarly be found in edematous states like cirrhosis of liver or congestive heart failure, the body sodium may actually be in excess.

On the other hand, the depletion of sodium caused by excessive losses of gastrointestinal fluids or by renal disease with salt wasting, indicates depletion of total body sodium which is reflected in a low serum sodium concentration. This condition of hyponatremia (low serum sodium) is accompanied by loss of water as indicated by rapid loss of weight. The hyponatremic states—one caused by dilution and overhydration and the other with true sodium-depletion in the body, can be differentiated by observing the changes in the body weight. The former is characterized by weight gain and the latter by weight loss due to dehydration.

Increased serum sodium (hypernatremia) is rather rare. Rapid administration of sodium salts or the hyperactivity of the adrenal cortex, as in Cushing's disease, may give rise to increased serum sodium concentration. The serum sodium level may also rise after administration of corticotropin (ACTH), cortisone, deoxycorticosterone or some of the sex hormones unless the sodium retention is masked by the concomitant retention of water. The rapid loss of water as it occurs in dehydration associated with diabetes insipidus, results in the development of the most common cause of hypernatremia (increased serum sodium). Excessive sweating may occasionally give rise to hypernatremia. The loss of fluid in excessive sweating exceeds the loss of salt to disturb the ratio of salt to water.

Adrenocortical insufficiency, as in Addison's disease, shock, due to loss of blood volume (in surgery, wounds or severe burns), prolonged vomiting (chloride loss) or diarrhoea (sodium loss) is characterized by increased sodium loss. Pregnancy has an ameliorating effect on

adrenocortical insufficiency (Addison's disease). This may be due to the production of steroid hormones which cause sodium retention. Placenta also has been found to elaborate hormones having sodium retaining effects. These hormones appear to be responsible for the retention of sodium and water in certain stages of pregnancy.

Besides the primary function in the maintenance of normal osmotic pressure relations throughout the body, of the normal state of acid-base balance and water balance and in its intricate role in gaseous transport, both sodium and potassium are important in maintaining muscle and nerve irritability at the proper level. They are antagonistic to calcium and magnesium ions; the ratios of various ions are therefore, of importance as well as the absolute amounts in which the ions are present in body fluids and cells. In blood plasma sodium and potassium chlorides have the outstanding function not only of keeping the globulins in physical solution, but also of regulating the degree of hydration of the plasma proteins, for maintaining proper viscosity of blood.

Gastric HCl is derived from the sodium chloride of the blood and the base in other digestive fluids, such as the pancreatic juice and bile, is obtained from blood sodium and potassium salts. The extent of the excretion of sodium depends on the amount of intake. Surprisingly small quantities, in rat as little as 0.1 per cent of sodium chloride in the diet, can maintain animals—providing perhaps the minimum amount necessary. Below minimum quantities, there are loss of appetite, retarded growth, disturbance of the reproductive function, and ultimate death.

Ingestion of large quantities of sodium chloride in the diet produces a syndrome in rate resembling · nephrosis characterized by sudden onset of massive edema, hypertension, anaemia and prolonged lipemia, severe hypoproteinemia, and azotemia. The degree of hypertension parallels with the amount of salt in the diet, the moderating effect of potassium on the blood pressure being observed only at the high levels of sodium chloride intake. A low sodium chloride diet has been suggested in the treatment of hypertension.

Potassium

Potassium resembles sodium in its ease of absorption and general metabolism but each element has very specific functions and these elements cannot replace one another. Potassium is found very largely in the cells of the body and sodium is widely distributed in the body fluids.

FUNCTIONS

The principal cation of the intracellular fluid is potassium. It is also an important constituent of the extracellular fluid, influencing the muscle activity notably the cardiac muscle. Like sodium in the extracellular

fluid, potassium influences the acid-base balance, maintenance of osmotic pressure and water retention within the cells.

REQUIREMENTS AND SOURCES

The actual requirement of potassium for the body is not definitely known. The element however, is so widely distr buted in foods, deficiencies are not likely to occur under normal conditions. About 4 g per day is the normal intake of potassium in food.

The distribution of potassium in body fluids or tissues is indicated in Table 6.15.

TABLE 6.15

Fluid or tissue	mg per 100 ml or per 100 g	meq per litre
Whole blood	200	50
Plasma	20	5
Cells	440	112
Muscle tissue	250–400	
Nerve ti sue	530	

METABOLISM

Compared to sodium, the element potassium occurs in the extracellular fluid in relatively in small amounts but changes in the concentrations of the extracellular potassium affect the activity of s ated muscles and paralysis of the skeletal, muscle and abnormalities in conduction and activity of the cardiac muscle take place. Potassium is excreted into the intestine but most of it is reabsorbed later The kidney is the principal organ of excretion for potassium. It is filtered in the glomeruli as well as secre.ed by the tubules. The acid-base balance and the adrenal cortex influence the excretion of potassium.

Hyperkalemia (increased serum potassium) does not occur normally because of the great capacity of the kidney to excrete potassium, even when potassium is administered in relatively large amoi nts, provided, of course, the kidney function is not impaired. This is not however, true when kidney function is impaired and urine production is inadequate. Before correcting the circulatory collapse, dehydration and renal insufficiency, potassium should not be given intravenously.

Using radioactive potassium, it can be shown that the element penetrates rapidly into most of the tissues of the body and only a small quantity is found in the plasma. Dogs grow poorly and develop paralysis on potassium-deficient diets. Administration of potassium salts corrects these abnormal symptoms.

HYPERKALEMIA OR INCREASED SERUM POTASSIUM

Renal failure, advanced dehydration or shock given rise to an elevated serum potassium level. In Addison's disease, associated with an adrenal cortex deficiency, the potassium concentration in serum is definitely increased. An interesting metabolic disorder, hyperkalemic paralysis, has been found in human beings, characterized by periodic attacks of weakness or para- lysis, associated with increased serum potassium concentration. The elevated serum potassium is corrected by giving desoxycorticosterone acetate (Doca). Excessive administration of potassium intravenously may also cause hyperkalemia.

The cardiac and central nervous system depression-symptoms are related to the elevated plasma potassium concentration and not to increases in intracellular levels. Bradycardia and poor heart sounds, followed by peripheral vascular collapse and ultimately, cardiac arrest constitute the symptoms of hyperkalemia. Electrocardiographic changes in heart signs are characteristic with elevated T waves, widening of the QRS complex, progressive lengthening of the P-R interval, and then disappearance of the P wave. Mental confusion, weakness, numbness, and tingling of the extremities, weakness of the respiratory muscles and a flaccid paralysis of the extremities constitute other symptoms commonly associated with increased concentration of extracellular potassium.

HYPOKALEMIA OR DECREASED SERUM POTASSIUM

Intravenous administration of potassium-free solutions, particularly in postoperative states may give rise to hypokalemic condition. Chronic wasting diseases with malnutrition, prolonged negative nitrogen balance, gastrointestinal losses and metabolic alkalosis may cause potassium deficiency. In most of these cases, the intracellular potassium is trans- ferred to the extracellular fluid, from where it is quickly removed by the kidney. Overactivity of the adrenal cortex such as in Cushing's syndrome or primary aldosteronism or injection of excessive quantities or corti- costeroids or corticotropin (ACTH) may induce potassium deficit, due to increased excretion of potassium caused by the adrenocortical hormones, particularly aldosterone.

Certain diuretic agents such as acetazolamide and chlorothiazide, cause an increased excretion of potassium in the urine. In such cases potassium supplementation has been recommended. Severe damage to the kidney is caused by prolonged potassium deficiency with secondary development of chronic pyelonephritis. The mitochondria in the collecting tubule is affected in potassium-depleted animals.

The potassium content of the myocardium becomes depleted during heart failure and intracellular potassium repletion occurs with recovery. Potassium depletion may occur in fully digitalized patients who are given diuretic agents. Such manifestations of digitalis toxicity

may be prevented or relieved by potassium administation.

Correction of dehydration and acidosis or alkalosis with water and sodium often brings about potassium deficits. 0.36 mM of potassium is retained with the storage of 1 g of glycogen. Potassium is quickly withdrawn from the extracellular fluid due to rapid glycogenesis resulting from treatment of diabetic coma with insulin and glucose. The resultant hypokalemia may be fatal.

Potassium is rapidly transferred into cells, lowering the concentration of extracellular potassium in familial periodic paralysis This is a rare disease.

Muscle weakness, irritability and paralysis. tachycardia and dilatation of, the heart with gallop rhythm are the symptoms of hypokalemia. Electrocardiogram changes are characteristic in hypokalemia with include-first a flattened T wave, later, inverted T waves with sagging ST segment and A-V block and finally cardiac arrest.

Until late in the process the potassium deficiency may not be revealed in lowered potassium concentration in the extracellular fluid. A lowered concentration of intracellular potassium in muscle biopsy is found when serum potassium level is normal. Thus the true status of potassium balance is not indicated by serum potassium level.

Correction of potassium deficit by oral route is preferred. The proportion of potassium to nitrogen in muscle is 3 mM to 1 g. Additional potassium is therefore required for storage of nitrogen as muscle protein. It has been estimated that 600 meq of potassium alongwith protein nitrogen are necessary to compensate a loss of 5 kg of muscle protein. It is for this reason administration of potassium has been recommended alongwith aminoacids, to the extent of 5 meq of potassium per gram of amino acid nitrogen.

Chlorine

In the form of sodium chloride, the chloride ion plays an essential role in osmotic pressure relationships and in maintaining the water content in the body as well as in acid-base equilibrium. Chloride is specially important in acid-base equilibrium in the blood by the action of the chloride shift. Chloride is also of special importance in the formation of hydrochloric acid in gastric juice.

REQUIREMENT AND METABOLISM

Chloride is present in the diet almost entirely as sodium chloride and the intake of chloride is satisfactory so long as sodium intake is adequate. Both intake and output of chloride are inseparable from those of sodium.

The chloride concentration of normal serum is 340-370 mg per 100 ml or 97-105 meq per litre. A solution of 0.9 g of sodium chloride per

100 ml. is isotonic with serum, which means that the chlorides are responsible for two thirds of the osmotic pressure of the blood.

The chloride shift is of importance in acid-base equilibrium. The chloride ion readily passes through the cell membrane, which, however does not permit the passage of sodium and potassium ions.

The metabolism of chlorine cannot be separated from that of sodium. About 10-15 g of sodium chloride is needed daily and the chlorine (as chloride) is as readily absorbed and metabolized as is the sodium (as sodium chloride). The excretion of both chloride and sodium in the urine drop to low levels on low-salt diets.

Deficiency of sodium, potassium or chlorine is not likely to occur under normal dietary conditions. However, excessive diarrhoea, pernicious vomiting or excessive prolonged sweating may bring about a sodium chloride deficiency. It is for this reason that extra sodium chloride in the form of salt water is given to subjects exposed to excessive heat (and the consequent sweating). In health the excess salt consumed is excreted by the kidney, since it is fairly completely absorbed from the intestine.

Abnormalities in sodium metabolism is generally reflected in chloride metabolism. Chloride deficit takes place when sodium losses are excessive as in diarrhoea, profuse sweating and in certain endocrine disturbances. Vomiting or in pyloric or duodenal obstruction causes the loss of chloride in excess of sodium. This results in a decrease in plasma chloride concentration and a compensatory increase in plasma bicarbonate (hypochloremic alkalosis). Hypochloremic (low serum chloride) alkalosis alongwith hypokalemia may occur in Cushing's syndrome or after the administration of excess of corticotropin (ACTH) or cortisone.

Besides the primary functions in the maintenance of normal osmotic pressure relations throughout the body, and the normal state of acid-base and water balance, the chlorides of sodium and potassium in the plasma have the outstanding function of not only keeping the globulins in physical solutions but also of regulating the degree of hydration of the

TABLE 6.16

Fluid or tissue	mg per 100 ml or per 100 g	m eq per litre
Whole blood	250	70
Plasma or serum	365	103
Cells	190	53
CSF	440	124
Muscle tissue	40	
Nerve tissue	171	

plasma proteins which is important for the maintenance of normal viscosity of blood.

Distribution of chloride in body fluids or tissues is indicated in Table 6.16.

Sulphur

Most of the sulphur is found in the protein molecule. It is present in all the cells of the body. The metabolism of sulphur like that of nitrogen, is very intimately associated with the metabolism of protein itself. The sulphur of the protein is essentially centred in the amino acids cystine and methionine. Practically all the sulphur intake is from these amino acids as they occur in various food proteins.

Glutathione, coenzyme A, insulin, thiamine, ergothionine, taurocholic acid, sulphocyanide, ethereal sulphates (esters of phenols and sulphuric acid), chondroitin sulphuric acid (in cartilage), and melanins (pigments of the body) are the other sulphur containing organic compounds found in the body. Blood and various tissues of the body contain small quantities of inorganic sulphates mainly of sodium and potassium.

The essential nature of sulphur in nutrition is evident from the fact that all animal species including man, require the sulphur—containing amino acid methionine. All body proteins and special protein molecules, such as enzymes and hormones are found to contain methionine. Many enzymes depend on a free-SH group for maintenance of their activity. The role of the sulphur-containing compounds in detoxication mechanisms and of the SH group in tissue respiration is important. A high-energy sulphur bond similar to that of phosphate plays an important role in metabolism. Sulphur as sulphate, is used in the body for the detoxication of a variety of molecules including indoxyl and phenol.

SOURCES AND METABOLISM

Two sulphur-containing amino acids, cystine and methionine, are the main sources of sulphur for the body. Elemental sulphur or sulphate sulphur it not known to be utilized. Organic sulphur is largely oxidised to sulphate in the body and are excreted as inorganic and ethereal sulphates (which has been discussed under urine). Utilization of sulphate in organic combination requires preliminary activation of the sulphate moiety.

DISTRIBUTION

Besides cystine and methionine, there are a number of other organic compounds containing sulphur. Blood and other tissues contain small amounts of inorganic sulphates with sodium and potassium.

Keratin, the protein of hair, hoofs, etc., is rich in sulphur-containing amino acids. The hairy animals such as the rat and the dog, require higher amounts of sulphur (cystine and methionine), than human beings. may be due to their additional hair.

The Trace Elements

A number of inorganic ions are essential for plant and animal life in rather minute quantities compared to the amounts of calcium and phosphorus required. They are referred to as trace elements. Iron, copper, cobalt, boron, nickel, manganese, molybdenum, aluminium, arsenic, zinc, silicon, iodine, bromine, selenium, and chromium constitute the trace elements. Most of them are essential either for plants or animals or for both.

Iron

Iron functions in the body almost exclusively in the processes of cellular respiration. It constitutes an essential component of many oxidation-reduction enzymes and is present in several other biologically significant proteins. The total iron content in a 70-kg man ranges between 4-5 g and the approximate composition of the iron-containing compounds in the human (70-kg man) is indicated in Table 6.17.

TABLE 6.17

Compound	Total in the body in gram	Iron content in gram	Per cent of total iron in body
Iron porphyrin (haem) compounds:			
Blood haemoglobin	900	3.0	60–70
Muscle haemoglobin	40	0.13	3–5
(myoglobin)			
Haem enzymes:			
Cytochrome. C	0.8	0.004	0.1
Catalase	5.0	0.004	0.1
Cytochrome, a, a_3, b.	—	—	—
Peroxidase	—	—	—
Non-iron porphyrin compounds:			
Transferrin (siderophilin)	10.0	0.004	0.1
Ferritin	2–4	0.4–0.8	15
Haemosiderin	—	—	—
Total available iron stores	—	1.2–1.5	—
Total iron		4–5	100

From S. Granick, *Bull. N. Y. Academy of Med.*, 30 (1954), p. 82.

From Table 6.17 it is evident that iron is a component of haemoglobin, myoglobin, and cytochrome and the enzymes catalase and peroxidase. Iron in all these compounds occurs as a component of porphyrin.

The circulating haemoglobin accounts for about 60-70 per cent of the total iron; myoglobin about 3-5 per cent and various haem-containing enzymes (catalase, peroxidase, and cytochromes) contain only small amount of iron. Thus almost 70 per cent of t 'e total iron occurs in the form of compounds involved in the processes of oxygen transfer or cellular respiration. The remainder of the iron in the body is almost entirely protein-bound and occurs in three forms, constituting the storage and transport forms of the mineral. These three forms are; transferrin, ferritin, and hae mosiderin.

TRANSFERRIN

About 120 micrograms (μ g) iron is present per 100 ml of plasma. It is associated with a β_1-globulin fraction of plasma proteins and is called transferrin, siaerophilin, o: most simply, the plasma iron-binding globulin. The plasma is partially unsaturated with respect to iron. Iron is taken up by the unsaturated iron-binding globulin on addition of iron salts to plasma and the process continues until no more iron can be bound. The quantitative method of estimating the plasma latent iron-binding capacity is based on this. Normal plasma iron-binding capacity in adults ranges between 200-300 μ g iron per 100 ml and normally only about one-third of the iron-binding capacity is saturated. The protein β_1-globulin binds two iron atoms per mole and has a molecular weight around 90,000. The iron is transported to tissues and storage depots by this protein.

The nature of the bond between the iron and protein the complex, transferring, is not known despite the fact that oxygen and carbon dioxide are required for the reaction with ferrous iron. The bond is very strong but can be weakened and iron removed at neutral pH in presence of reducing agents. This indicates that the bond with ferrous iron is much weaker than with the ferric iron which forms when iron is added to the protein.

FERRITIN

An unusual iron-protein complex called ferritin constitutes about 15 per cent of the nonporphyrin iron. It can be obtained in crystalline form containing 17 to 25 per cent of iron by dry weight. It is found in the iron storage depots, liver, spleen, and bone marrow as well as in other organs in smaller amounts. These tissues contain ferritin and haemosiderin. When tested in the ultracentrifuge, ferritin in solution behaves like a mixture of molecules of varying size. The smallest of these molecules has a molecular weight of 465,000, and is essentially without iron. Larger quantities of iron are present in the molecules of

greater size, when treated with reducing agent at acid pH, the iron of ferritin can be removed completely by dialysis, a colourless protein, apoferritin, is left over. The molecular weight of apoferritin is 465,000. Apoferritin behaves like a single molecular species under ultra-centrifuge. The bulk of the ferritin iron in the protein molecule appears to be in the form of colloidal micelles of iron hydroxide-iron phosphate.

The bulk of the ferritin iron is in the ferric state and is not dialysable at neutral pH but a small portion exists in the ferrous state at or near the surface. In presence of iron binding agents like transferrin, the ferrous iron can be removed at neutral pH. The enzyme xanthine dehydrogenase can bring about the reduction of surface ferric iron to the ferrous form. The reaction is:

$$\text{xanthine} + \text{enz.ox} \rightarrow \text{Uric acid} + \text{enz.red.}$$

$$\text{Enz.red} + \text{Ferritin.Fe}^{+++} \rightarrow \text{Enz.ox} + \text{ferritin.Fe}^{++} + \text{H}^{+}$$
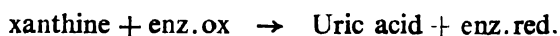
The reduced flavoprotein enzyme is reduced by xanthine, which is oxidized to uric acid. The reduced enzyme is then oxidized by ferric-ferritin, which in turn is reduced to ferrous-ferritin. The ferrous iron of ferritin is dissociated to combine with transferrin:

$$\text{Ferritin.Fe}^{++} \rightleftharpoons \text{Ferritin} + \text{Fe}^{++}$$

$$\text{Fe}^{++} + \text{transferrin} \rightarrow \text{Fe}^{+++} \text{transferrin.}$$
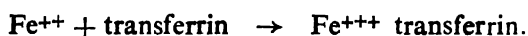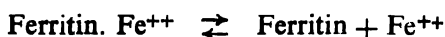
The release of iron from tissue stores may be explained as the normal mechanism of the action of xanthine dehydrogenase.

HAEMOSIDERIN

Ferritin and haemosiderin are present in liver, spleen and bone marrow. Radio iron given parenterally or orally is stored in both compounds, and mobilized iron is derived from both fractions. The two forms appear to be functionally indistinguishable and may represent only different physical forms. The compounds under normal conditions together with smaller stores may constitute about one-fifth of the total body iron. They are drawn upon in time of iron need for the production of haemoglobin and other haem-containing molecules. Haemosiderin appears as insoluble iron-protein granules in histological sections of liver and spleen. The chemical nature of these particles is obscure. Excessive deposits of these granules occur throughout the body in the disease known as haemo-siderosis. These probably represent the storage form of excess iron in equilibrium with ferritin.

SOURCES AND AVAILABILITY OF IRON

The need for iron in the human diet varies greatly at different ages and

under different conditions.   When the demand for haemoglobin formation is increased as during growth, pregnancy and lactation, additional iron is required in the diet.   Iron deficiency in adults is not likely to occur unless some loss of blood takes place.   Malabsorption from the gastrointestinal tract may cause iron dificiency.

The average loss of blood during a menstrual period is 35 to 70 ml representing a loss of 14 to 28 mg of iron.   Diet provides this amount of iron easily.   The dietary requirement for iron is almost negligible in healthy adult male or in healthy women after menopause.

Haemoglobin formation requires the presence of traces of copper. The requirements of daily amounts of iron are:

Children between 1-3 years of age	15 mg
Children between 3-10 years of age	10 mg
Older children and adults:	
Males between 10-12 years of age	10 mg
Males between 12-18 years of age	18 mg
Males after 18 years of age	10 mg
Females between 10-55 years of age during pregnancy and lactation	18 mg
Females after 55 years of age	10 mg

The important dietary sources of iron are organ meats, liver, heart, kidney, and spleen.   Egg yolk, whole wheat, fish, nuts, dates, figs, beans, spinach, molasses and oatmeal constitute other good sources.

ABSORPTION

Unlike other inorganic constituents of the body, very little dietary iron is absorbed by the adult and very little is excreted in the urine.   Most of the dietary iron is eliminated with the faeces.   Very little dietary iron is absorbed under normal conditions and the amount excreted in the urine is minimal, most of it being eliminated in the faeces.   The absorption of iron from the intestine is controlled in such a way that its accumulation in the tissues in toxic amounts does not occur normally since there is no other way to excrete excess iron.

The ordinary diet contains about 10-20 mg of iron but less than 10 per cent of the intake is absorbed.   This indicates that the iron present in the body must be used over again.   Normally less than 2 mg of iron is absorbed per day.   Part of the iron present in foodstuffs is converted into its salts by gastric HCl and then gets reduced by various food reducing agents such as glutathione, ascorbic acid, and many sulphhydryl (—SH) groups of protein amino acids into the ferrous state.   Most of the iron in foods occurs in the ferric state (Fe^{+++}) either as ferric hydroxide or as ferric organic compounds.   Iron in the ferrous form (Fe^{++}) is more soluble and the ferrous ion is absorbed especially in the

upper duodenum. The concentration of ferritin increases in the same areas of the intestine as the absorption of iron occurs.

Iron absorption, apparently is little influenced by gastric acidity, its absorption is not necessarily altered in achlorhydria (absence of hydro-chloric acid in gastric juice). Iron enters the mucosal cells in the ferrous form and is stored in ferritin as ferric hydroxide. More iron from the intestine is absorbed into the mucosal cells as the iron content in ferritin decreases to a critical point. When iron stores are low increased absorption takes place and excessive iron intake decreases its absorption. Experiments using radioactive iron compounds indicate that some sort of braking mechanism exists in the intestinal mucosa which does not permit the absorption of iron until there is body need for iron as may happen in haemorrhage with iron loss. The mucosal block is lifted, leading to increased iron absorption when the iron stores (ferritin) are partially depleted. The regulating mechanism may be in the apoferritin as iron acceptor.

Iron absorption is impaired by subtotal or total removal of the stomach or by surgical removal of a considerable amount of the small bowel. Various malabsorption syndromes, such as steatorrhea, may also cause diminished iron absorption.

The absorption of iron may increase 2 to 10 times normal in iron-deficiency anaemias. Pernicious anaemic or hypoplastic anaemia also causes increased iron absorption. Iron absorption is decreased by diet high in phosphate as compounds of iron and phosphate are insoluble. Low phosphate diet, on the other hand, increases iron absorption markedly. Phytic acid present in cereals, and oxalic acid interfere with the iron absorption.

All of the iron in foods is not available to the body. Nutritionally available iron is determined by measuring the amount that will react with α, α-dipyridyl reagent.

The mucosal block appears to regulate the amount of ferrous iron entering the cell. The ferrous iron is oxidized to the ferric state within the cell and combines with a protein, apoferritin, to form an iron-containing derivative, ferritin, which contains 23 per cent of iron by weight.

The binding capacity of apoferritin for iron limits the absorption of iron. The apoferritin content of the mucosal cells is normally low but it has been demonstrated that the administration of iron salts stimulates the synthesis of apoferritin to a considerable extent.

IRON STORAGE AND TRANSPORT

Iron enters the blood from mucosal cell ferritin when the blood level of transport iron is decreased. It is associated with a β_1⁻ globulin fraction of plasma proteins known as transferrin or siderophilin. Transferrin is a glycoprotein containing hexose, hexosamine, sialic acid and probably fucose.

The iron entering the blood stream from the intestine is mostly in the ferrous state (Fe^{++}). It is readily oxidized to ferric state (Fe^{+++}) in the plasma to get incorporated into the specific iron-binding protein, transferrin forming a red ferricprotein complex, each molecule of the protein binding two atoms of Fe^{+++}. Oxygen is required for the formation of the red complex.

METABOLIC CYCLE OF IRON

Use of radio active ^{59}Fe has aided in understanding the movement of iron within the body. It does not upset the normal iron concentration in the body fluids when injected in tracer doses, yet its course can be followed. [59]Fe combines with the iron binding globulin when injected into the plasma and disappears at a rate whose half life in the normal human is 100 minutes. Most of this iron goes to the bone marrow and smaller amounts to the liver and spleen. The iron taken up by the bone marrow finds its way into the haemoglobin of newly born red cells which are soon found in the circulation.

The dying red cells are phagocytized by the reticulo-endothelial portion of the liver, spleen and bone marrow. The haemoglobin is degraded to amino acids, bile pigments and iron. The iron is incorporated into tissue ferritin, as well as liberated into the plasma to continue its cycle. The tissue ferritin, mostly liver, constitutes a convenient storage form of iron, taking care of excess iron not required immediately for haemoglobin synthesis and releasing iron for maintaining a plasma concentration suitable to the needs of bone marrow. Catalase and cytochromes are the other non-proteins synthesized in addition to haemoglobin. The turnover of these proteins in the body can be measured by following the incorporation and release of its radioactive iron.

Ferritin is found in the human placenta which suggests that the release of iron from ferritin regulates the transfer of iron from the maternal plasma to foetal plasma so as to supply iron for haemoglobin synthesis (alongwith catalase and cytochromes) in the foetus.

The level of iron in the plasma is the result of a dynamic equilibrium. The factors influencing the equilibrium, include the rate of breakdown of haemoglobin, uptake by the bone marrow in connection with the red blood cell synthesis, removal and storage by the tissues, absorption from the gastrointestinal tract, and the rate of formation and decomposition of transferrin. Use is made of isotopic ^{59}Fe in the studies of iron turnover which indicates utilization of about 27 mg per day, 75 per cent of which for haemoglobin formation. Breakdown of red blood cells accounts for about 20 mg, newly absorbed iron contribute a very small amount and the remainder comes from the iron stores. The mobilization of iron from the storage depots is a slow process.

ALTERATIONS IN IRON METABOLISM

Anaemias represent the most common derangement of iron metabolism with abnormally low concentration of haemoglobin in the circulation. The anaemias may be due to iron deficiency in the diet, known as nutritional anaemias. Iron deficiency anaemias are of the hypochromic microcytic type. Inadequate intake (a high cereal diet, low in meat) or inadequate absorption (gastrointestinal disturbances—diarrhoea, achlorhydria, steatorrhoea, or intestinal disease, etc.), as well as excessive loss of blood (haemorrhage) may result in iron deficiency.

Iron-deficiency type of anaemia can be successfully treated by daily addition of ferrous sulphate to the diet in case the absorption is adequate. An iron preparation (iron dextran) is used for intramuscular injection in patients unable to tolerate or absorb iron by oral administration. Over saturation of the tissues may occur by parenteral administration of iron preparation resulting in haemosiderosis; hence caution is necessary in such administration. Loss of ability to absorb vitamin B_{12} present in the diet, may also give rise to iron deficiency anaemia. This is pernicious anaemia, in which lack of vitamin B_{12} results in the loss of ability to utilize iron for haemoglobin synthesis. Chronic infectious diseasees or a deficiency of vitamin pyridoxine may also cause anaemia.

Temporary loss of blood (red cells) causing anaemia brings about the release of iron from ferritin stores and acceleration in the synthesis of red cells in the marrow for the early correction of the anaemia. When the ferritin stores are lowered, the mucosal block to iron absorption is lifted and more iron enters the body from dietary source. Young children and infants are especially prone to anaemia due to nutritional deficiency in the diet.

Normally one half of the radioactivity of ^{59}Fe given intravenously in tracer doses disappears exponentially from the circulating blood in 100 minutes. The half life period in haemolytic anaemias with hyperplasia of the erythroid tissue and polycythemia vera, comes down to 11 to 30 minutes. The disappearance time in aplastic anaemia is prolonged to 250 minutes. The uptake of iron in an iron-deficiency type of anaemia is accelerated in the erythrocytes. In aplastic anaemia, it is diminished.

An excess amount of iron may accumulate in the tissues because of the absence of an excretory pathway for iron. This happens in aplastic or haemolytic anaemia where many blood transfusions have been given over a period of years. This is known as haemosiderosis, which may be accompanied by a bronzed pigmentation of the skin, called haemochromatosis, presumably due to the toxic effect of the unbound iron in the tissues. Liver damage with signs of cirrhosis, diabetes and a pancreatic fibrosis—a condition called bronze diabetes.

The anaemia caused by pyridoxine deficiency is corrected by giving the vitamin. The plasma iron is abnormally high in pyridoxine deficiency unlike that in anaemia of chronic infection.

Polycythemia, an increase in blood haemoglobin above normal levels is associated with an overproduction of erythrocytes despite life span of the red cells remaining normal. High altitudes or an atmosphere low in oxygen content may induce polycythemia. It may be that anoxia stimulates the erythropoietic tissue of the bone marrow. Administration of cobalt salt also induces this condition, by an unknown mechanism.

Copper

Copper is present in all living matter—plants and animals. It may be associated with the formation of chlorophyll in plants. Copper is associated with haemoglobin formation through a mechanism still obscure. It appears to be concerned in young erythrocyte formation. Copper deficiency in animals results in fewer red cells but the haemoglobin concentration is not changed. No specific role of this essential element in haemoglobin synthesis has been found.

Several naturally occurring organic substances contain copper. Haemocyanin, a copper-protein complex found in the blood of certain invertebrates, is one of them. Haemocyanin functions as an oxygen carrier in crab, spider, snail, etc., similar to haemoglobin in man. Copper is a constituent of certain oxidizing enzymes or is essential in their activity. Examples are ascorbic acid oxidase, polyphenoloxidase, cytochrome oxidase, catalase, tyrosinase and uricase. They are copper-protein complexes. The red blood corpuscles of mammals have been found to contain a copper-protein compound, called haemocuprein. From liver another copper-protein compound, hepatocuprien, has been isolated. Cerebrocuprin, a copper-protein complex, has been isolated from human brain. The normal adult human red blood cells contain about 30 to 36 mg of erythrocuprein per 100 ml of packed cells. The erythrocuprein accounts for most if not all of the copper in the red cell. Ceruloplasmin is the copper-binding protein of the serum or plasma. Normal plasma

TABLE 6.18

Protein	Molecular weight	Copper per cent	Substrate
Butyryl coenzyme A	1,20,000	0.35	Saturated acyl coenzyme A
Dehydrogenase	2,20,000	—	Derivatives of fatty acids
Uricase	1,10,000	0.06	Uric acid
Tyrosinase	1,00,000	0.25	Tyrosine, dopa
Ceruloplasmin	1,51,000	0.34	Paraphenylene diamine
Hepatocuprein	25,000	0.34	?
Haemocuprein (erythrocuprein)	35,000	0.34	?
Milk copper protein	—	0.19	?

contains about 30 mg of ceruloplasmin per 100 ml. It acts in vitro as an enzyme—a polyphenol oxidase. Ceruloplasmin may also function in oxidation of plasma iron for the formation of transferrin-bound iron.

The copper content of some of the proteins and enzymes in mammalian tissues is indicated in Table 6.18

REQUIREMENT AND SOURCES

The daily needs of copper have been estimated to be about 2 mg. Much of its excretion is through the bowels. Copper is widely distributed in foods and a dietary deficiency is not likely to occur in human beings except in infants fed on milk diet exclusively. Cow's milk contains from 0.09 to 0.17 mg of copper per litre. The adult human body contains 100 to 150 mg of copper; about 64 mg are found in muscles, 23 mg in the bones, and 44 mg in the liver, which contains a higher concentration of copper than any of the other organs. The copper content of the whole blood in adults is over 100 μg per 100 ml. The value may become very low in nutritional anaemia. Copper appears to be evenly distributed between cells and plasma.

The copper content in the foetal liver is five to ten times higher than that in the adult liver. Both blood cells and serum contain copper, the copper content of blood cell is constant but that of the serum is highly variable with an average of 90 μg per 100 ml. The serum copper occurs in two distinct fractions. The fraction reacting directly with diethyldithio-carbamate, used in colorimetric estimation of copper, is called direct-reacting copper. This copper is loosely bound to albumin representing probably the copper in transport. The serum copper present in this form is relatively little. Most of the copper in the serum, about 96 per cent, is bound to an alpha globulin contained in the Cohn fraction iv–1. This copper from the globulin bound form is required to be freed first by treating serum with hydrochloric acid so that it can react with the copper reagent. This is the indirect reacting copper.

The copper content in different foods is indicated in Table 6.19.

TABLE 6.19

Substance (per kg)	Copper in mg
Liver	44.1
Nuts	11.6
Legumens	9.0
Cereals	4.7
Fruits	4.2
Poultry	3.0
Fish	2.5
Green legumens	1.7
Leafy vegetables	1.2

Nutritional deficiency of copper has not been positively demonstrated in man, sprue or nephrosis has been suspected to cause its deficiency. In infants copper has been found to be a beneficial adjunct to iron therapy in the treatment of nutritional anaemia. A hypochromic microcytic anaemia characterized by low levels of serum copper and iron and by edema has been reported to occur in infants. The syndrome is easily relieved by iron therapy.

A copper-deficiency syndrome, including ᴖnaemia, has been found to occur in sheep pasturing in copper-deficient grazing land. The anaemia responds to copper therapy.

METABOLISM

Copper is not readily excreted in the urine as it is largely bound to protein in the plasma. Most of copper is lost in the faeces through the intestine.

Copper-deficient diet causes loss of weight and death in experimental animals with the development of severe hypochromic microcytic anaemia. This is not the cause of death as the iron-deficiency anaemia of equal proportions is not fatal. Copper has therefore, been suggested to play a role in the body in addition to its function in the metabolism of red cells. The activity of oxidation-reduction enzymes of the tissues, such as the cytochrome system, may be due to the additional role of copper. Copper and iron metabolism appear to be related. The movement of iron from the tissues to the plasma results in copper deficiency with hypoferremia. Absorption of iron from the gastro intestinal tract is increased by the presence of copper.

Deficiency of copper in the diet of young dogs causes a bone disorder, characterized by abnormally thin cortices, deficient trabeculae, and wide epiphyses. Fractures and deformities take place in many of the animals. Anaemia develops and the hair turns gray.

Wilson's disease or hepatoleuticular degeneration, is associated with abnormalities in copper metabolism. Large amounts of copper are found in the liver and lenticular nucleus of the brain. Urinary excretion of copper is excessive resulting in low levels of copper and of ceruloplasmin in the plasma. This disease is associated with a generalized aminoaciduria.

Absorption of copper from the intestine is considerably increased in Wilson's disease, with the result that copper accumulates in the tissues and appears in the urine. Cirrhosis may develop with excessive copper deposition in the liver. Renal tubular damage, leading to increased urinary excretion of amino acids and peptides, and of glucose as well, results from an accumulation of copper in the kidney. Presence of abnormal amounts of unbound copper accounts for the excretion of copper in the urine. Within 24 hours of ingestion copper is bound to ceruloplasmin under normal conditions, but the copper is still associated with

the albumin fraction in Wilson's disease, with the result that the direct-reacting serum copper fraction is not reduced in these patients, on the contrary it may increase. The total serum copper may remain normal or slightly reduced. Hypocupremia does not appear to have any diagnostic significance, and may occur in a variety of conditions.

Iodine

Courtois discovered iodine in seaweed and it was postulated as early as 1820 that iodine was a curative for goiter. Iodine is obtained largely from food and, to some extent, from salt and water. The iodine content of the nearby soil as well as its fruits, grains, grasses, and vegetables is measured at times, by the amount of iodine found in drinking water of the region.

In 1896 Baumann discovered that the thyroid gland is far richer in iodine than other tissues. This led to the experiments on the relation of dietary iodine and thyroid function and corroborated the earlier postulates of higher incidence of thyroid enlargement among peoples in areas where the soil and water are low in iodine.

About 25 mg of iodine is present within the body of a man weighing 70 kg and 15 mg of which is found in the thyroid. Iodine is an important precursor in the formation of thyroxine. The iodine-containing hormone cannot be fabricated without available iodine.

The iodine content of the drinking water and the incidence of goiter show an inverse relation. The drinking water is not however an important source of iodine in the diet but it serves as an index of the iodine in the vegetation. In certain regions the water and the food grown on that soil contain less iodine than is necessary for normal well-being which results in the appearance of goiter in its various stages. The simplest and a very effective treatment is the incorporation of a small quantity of iodine, in the form of sodium iodide or potassium iodide, in the common salt—one part of sodium iodide in 100,000 parts of sodium chloride is sufficient. This is called 'iodized salt'.

The daily requirement of iodine has been estimated to range from 100 to 200 mg. The concentration of iodine in sea water is low but sea life, such as algae, fish, oysters concentrate iodine and are good sources of iodine. The need for iodine is increased in adolescence and in pregnancy.

It was estimated in 1962 that 200 million people in the world suffer from some degree of goitre.* The great majority of these are due to iodine deficiency. Goitrogenic chemicals such as thiocyanates found in foods of the cabbage family, account for some by inhibiting iodine uptake by the thyroid. Some cases of goitre are due to thiourea compounds, present in mustard, which inhibit iodination required in the formation of the hormone.

*J. Matovinovic, *J. Am. Med. Wom. Ass.*, 17 (1962). pp. 427, 495, 571, 646.

Probably all the cells of the body contain iodine. The concentration of iodine in the thyroid gland is remarkable, which accounts for as much as 70 to 80 per cent of the total iodine content in the body, amounting to only about 0.2 per cent of body weight.

Use of radioactive (^{131}I) has shown that iodine from the blood stream is rapidly transported to the thyroid where it is incorporated in organic molecules quickly in the formation of th roxine. Administration of radioiodine (^{131}I) often serves as a means for assessing thyroid function. The iodine content in blood of normal man varies, depending somewhat on intake. It ranges from 3 to 20 μg per 100 ml of serum. The various fractions of blood iodine levels serve as diagnostic aid in thyroid disease. It is believed that protein-bound iodine of serum represents principally the circulating thyroid hormone. The protein-bound iodine of serum is reported to be : *

in normal children = 4.0 to 7.0 μg per 100 ml of serum
in normal adults = 6.0 to 8.4 μg per 100 ml of serum
in cretinism
(juvenile hypothyroidium) = 1.8 to 3.0 μg per 100 ml of serum
in thyrotoxicosis
(hyperthyroidism) = 9.2 to 14.5 μg per 100 ml of serum

Iodinated proteins have been used as a dietary supplement in animal husbandry. It is possible by judicious use of iodinated casein, to stimulate increased milk production in cows, improve libido and fertility in inactive bulls, induce growth rate in young pigs, and possibly improve egg production in chickens. The physiological effects of iodinated protein are largely due to their thyroxine content.**

The average daily urinary excretion of iodine by normal adults has been estimated at 50 μg. The functions and metabolism of the iodine-containing thyroid hormone have been discussed before in detail.

Fluorine

Fluorine is present in various tissues of the body, particularly in bones and teeth. Normal bone contains 0.01 to 0.03 per cent of fluorine and dental enamel. 0.01 to 0.02 per cent. Plant growth is not known to require fluorine although it is practically always present in soil.
The essential nature of this element in animal nutrition is rather conflicting, as no diet has so far been devised which is free from fluorine. Fluorine seems to improve tooth development when present in very small amounts. Mottled enamel, a defect in teeth, is caused by a slight excess.

*N.B. Tallot, *et al.*, *J. Biol. Chem.*, 153 (1944), p. 479.
**J. Meites: *Milk: The Mammary Gland and its Secretion* (London: Academic Press, 1961), p. 321.

It has been attributed to the fluorine in the drinking water. Mild mottling of the teeth occurs in less than 2 per cent of the children living in areas where the fluorine content of water ranges from 0.6 to 1.2 parts per million (ppm). With the increase in the fluorine content in water, severity and incidence of mottling increase. The amount must be in excess of one part per million. Such teeth show chalky white patches and the enamel is frequently pitted and corroded. Imperfect classification is revealed on histological examination. Mottled enamel is confined primarily to the permanent teeth and develops only during their formation. Mottling does not occur in adult enamel. The incidence of dental caries is found to be markedly reduced in areas of endemic mottling indicating fluoride to be associated with caries inhibition in human beings.

The incidence of detail caries in children is much lower when the levels of fluorine in water are above 1 ppm. Mottled enamel is called fluorosis. Dental caries and lack of fluorine appear to be linked. The amount of fluorine in the water produces mottled enamel, gives some protection against dental caries, which is more prevalent where the drinking water contains a trace of the element. The incidence of osteoporosis in man is materially reduced by long-term consumption of fluoridated water. Osteoporosis is characterized by softening of bone as a result of excessive absorption of bone elements. Sodium fluoride (NaF) in doses 1 to 3 mg per day of fluoride ion in water, or as a tablet, has been found to be of great benefit in reducing the incidence or in alleviating postmenopausal osteoporosis. Bone pain in many cases is reduced markedly and calcium phosphorus balances are reversed from negative to positive.*

Fluorine as fluoride, given in relatively large doses, is quite toxic. Administration of 8 to 9 mg of fluorine per kg of body weight produces loss of appetite, disturbed osseous metabolism, and fatty degeneration in cattle. Studies in oxygen uptake suggest interference with cellular metabolism. Fluoride is a poison for some enzyme systems. It inhibits specifically, the conversion of glyceric acid to pyruvic acid by enolase in anaerobic glycolysis.

Bromine

Bromine occurs regularly in plant tissues as most soils and water contain small amounts of the element. It is present in various tissues of the body. The bromine content of blood in man ranges between 0.15 to 0.55 mg per 100 ml normally. Significant amounts of bromine have been detected in the brain (hypophysis). The bromine content in blood is markedly lowered in manic-depressive psychoses.

About 1 mg of bromine is present in every gram of chlorine in ordinary salt. A slight growth response to NaBr in chicks has been

*D.S. Bernstein, *Post-grad. Med.*, 34 (1963), p. 407.

found in chicks under controlled feeding conditions, which however, is of academic interest only at present.

Manganese

Manganese occurs in plant and animal tissue and appears to belong to the essential group. The essential nature of the element nutrition has long been known. It is added routinely to the nutrient solution employed in hydroponics—soil-less plant growth.

Ordinary daily diet contains about 4 mg of manganese. This amount is in excess of that required by man. This element is essential as indicated by animal experiments. Its deficiency in man has not been demonstrated. Rats reared on manganese-deficient diets fail to suckle their young and males develop testicular degeneration leading to complete sterility. Growth is poor, bone formation abnormal, interference in haemoglobin regeneration and blood serum phosphatase is markedly elevated in rats reared on purified diets providing only 5 μg of manganese daily. Liver arginase is decreased. Manganese deficiency produces characteristic symptoms in other species. Its lack gives rise to perosis (slipped tendon disease) in chickens and other fowl.

The total manganese content in the body is about 10 mg, the kidney and the liver being the chief storage organs for the element. Liver accounts for about 0.17 mg and kidney 0.087 mg per 100 g of tissue. The managanese content in blood ranges between 0.004 to 0.020 mg (4 to 20 micrograms) per 100 ml.

Most of the manganese is excreted with the faces. Bile plays an important role in the intestinal excretion of the element as determined by experiments with isotopic manganese—about 50 to 75 per cent of which is carried by the bile. Very little manganese is excreted in the urine.

The functions of manganese are not precisely known. In vitro, manganese as Mn^{++} activates a number of enzymes such as arginase, phosphoglucomutase, hexokinase, isocitric dehydrogenase, pyprophosphatase, various decarboxylase, and cholinesterase. Other bivalent ions may be effective activators of certain enzymes but Mn^{++} may be specific for arginase. Deficiency of manganese causes a decrease in liver arginase, which increases substantially on addition of manganese salts to liver preparations from deficient animals.

β_1-globulin of human plasma binds manganese which is named transmanganin as distinct from the iron-binding protein, transferrin. The manganese content in blood is equally divided between cells and plasma. The element is concentrated in mitochondria of cells.

Average daily diet contains 12 to 20 mg of manganese. The manganese content in foods varies considerably. Beet tops, blue-berries, pineapple and wheat bran contain 100 to 200 mg per kg of dry material. Fruits

have less than 15 mg per kg. Fish or beef have little or none. Cow's milk contains about 0.03 mg of manganese per litre and eggs about 0.01 to 0.02 mg per egg. Spontaneous deficiency of the element in man is not likely to occur because of the universal distribution of manganese in plant and animal tissues, except under extraordinary conditions.

Cobalt

Cobalt is a constituent of vitamin B_{12}. It affects blood formation. Cobalt has been successfully used in the treatment of a nutritional anaemia in cattle and sheep reared on cobalt poor diet. Cobalt is utilized in the synthesis of vitamin B_{12} by the microorganisms in the rumen of these animals. Deficiency of cobalt causes a decrease in vitamin B_{12} supply resulting in anaemia. Anaemias in children have been reported to be successfully treated with cobalt. Administration of cobalt in rats produces polycythemia an excess of red blood cells, which is also produced in human subjects by the administration of cobaltous chloride.

Kidney is the chief organ for the elimination of the element almost completely as revealed by isotopic cobalt experiments. The production of erythropoietin is stimulated by cobalt. Cobalt accounts for about 4 per cent in vitamin B_{12} by weight and a deficiency of cobalt in ruminants gives rise to vitamin B_{12} deficiency.

Dietary cobalt is needed therefore by ruminants for supplying the required vitamin B_{12} as the element is involved in the synthesis of the vitamin by the microorganisms in the rumen. Oxidative metabolism of propionate has been found to depend on a vitamin B_{12} coenzyme.

Zinc

Zinc occurs in all naturally growing materials. It is an essential element for plant growth and its deficiency causes various plant diseases. Zinc is also essential in animal nutrition. Zinc-deficiency in rats causes poor body and fur growth. The catalase activity of kidney and liver is decreased in mice by zinc deficient diet.

Zinc is a constituent of crystalline insulin although zinc-free insulin preparations are available. The enzyme carbonic anhydrase, vital in many animal species, contains zinc. It is essential to the activity of carbonic anhydrase and thus to life in animals and man. Other enzymes such as dehydropeptidase, phosphatase, uricase, alcohol dehydrogenase, glutamic dehydrogenase, and several pyridine nucleotide dehydrogenase appear to contain zinc.

The zinc content of the pancreas in diabetic patients is about one-half the normal amount indicating that the element may be concerned with the storage and utilization of insulin. The whole blood contains 6.6 to 8.8 μg

of zinc per ml, plasma little over 1 µg per ml and erythrocytes 12 to 18 µg per ml, where it occurs mostly as carbonic anhydrase. Very small quantity of zinc is found in leukocytes from the blood of normal human subjects $(3.2 \pm 1.3 \times 10^{-10}$ µg per million cells) where the metal is protein-bound to the extent of 3 mg of zinc per gram of protein. These cells do not contain any carbonic anhydrase. The zinc content of white blood cells in human leukemia is however reduced to 10 per cent of normal amount.

Zinc is considered as an essential element but little is known about its requirement by human subjects. Average daily diet contains 12 to 20 mg of zinc. Zinc deficiency is unlikely to occur in man because of its wide distribution in foods of both plant and animal origin, except under abnormal circumstances.

Zinc content is high in oysters, liver, wheat germ, yeast, and lettuce. Milk contains only 2 or 3 mg of zinc per litre. The normal serum contains 120 ± 19 µg of zinc per 100 ml, which is reduced to 66 ± 19 in patients with Laennec's cirrhosis with increased urinary excretion of zinc. Certain enzymes require zinc for their activation. Significant biochemical changes occur in the metabolism of the substrates affected by the concerned enzyme. Alcohol is one such substrate. Alcohol dehydrogenase is a zinc-protein and continued use of alcohol may alter the enzyme in some way leading to its degradation and to zincuria. Much of the element is excreted by the intestinal tract. Radioactive zinc accumulates in the mucosa of the intestine and in the pancreas and the liver.

Aluminium

Aluminium is widely distributed in plant and animal tissues. There is no evidence to indicate that the element is essential in human nutrition and its role in physiology is still obscure. Rats reared on a mineralized milk diet showed no significant growth differences on addition of as little as 1 µg of aluminium per day. Diets with large amounts of aluminium are reported to cause rickets in rats by interfering with the absorption of phosphates.

The average human diet contains 10 to over 100 mg of aluminium. Very small amount of aluminium is obtained from food and from the cooking utensils; it can also be added to the diet as sodium aluminium sulphate in baking powder and as alum.

Absorption of aluminium from the intestine is very poor-about 100 µg per day. Most of the ingested aluminium is excreted in the faeces. The total aluminium content in the body ranges between 50 to 150 mg.

Boron

Boron has been recognised as essential in plant life and growth. There is

no clear evidence to suggest that animals normally need boron. Traces of the element are found in animal tissues. The growth of rats is not impaired on diets as low as 0.6 μg of boron per day. Boric acid is rather widely used in the treatment of extensive burns in human beings but it has a cumulative poisonous effect.

Molybdenum

Increased dietary molybdenum produces deleterious effects in cattle and sheep which respond dramatically on administration of copper sulphate by mouth. This has led to the postulation of a possible relationship of molybdenum to copper and other trace elements in nutrition. The response to molybdenum toxicity varies markedly with species variations. The most susceptible species are cattle and sheep, horses and pigs the least. The deleterious effects of excess molybdenum are decreased or even removed by dietary copper sulphate. This is partly due to the resulting lowered blood molybdenum levels.

Toxic levels of molybdenum have been found to bring about changes in enzyme activity in tissues from rats. The activity of the liver sulphide oxidase is depressed. Traces of molybdenum are necessary for the activity of the flavoprotein enzyme, xanthine oxidase—for the deposition and maintenance of normal levels of the enzyme in the intestine and liver of the rat. The element is actually a part of the enzyme molecule as has been found in highly purified preparation of the enzyme obtained from milk. Another flavoprotein enzyme, liver aldehyde oxidase, which catalyzes the oxidation of aldehydes, also contains molybdenum.

The molybdenum toxicity may be partly due to a poisoning effect of the accumulation of sulphide in the liver. Dietary copper prevents some of the disorders and some of the symptoms may result from insoluble copper sulphide in the tissues leading to copper deficiency in rats. Comparable relations in human nutrition are not known as yet.

Selenium

The forage crops concentrate soil selenium more than cultivated crops and crop plants growing in close proximity to seleniferous forage plants may themselves become highly toxic. Selenium when present in the diet above 5 to 15 parts per million (ppm) is highly toxic to animals particularly to ruminants maintained in pastures in selenium rich soil. Selenium improves growth in sheep and prevents several diseases in sheep and other animals when present below 3 ppm.

Selenium in very small amounts may be an essential factor in tissue respiration. Interest in selenium has recently been aroused by the discovery of a potent, metabolically active, selenium-containing compound called

'factor 3' which has been found to protect the liver against fatty infiltration and necrosis. This action of selenium may be related to that of vitamin E, as the two substances appear to act synergistically in curing the hepatic disease and certain muscle disorders induced in animals. The function of selenium involved is probably that of a cofactor for enyme systems related to cell oxidation. This may prove to be the role for this trace element in human nutrition also.

Cadmium

Presence of cadmium in traces in body tissues has been known for sometime. In 1960 cadmium was isolated as a definite component of a metal containing protein. This protein, metallothionein, found in the renal cortex of the horse, contains cadmium, zinc, and sulphur. The significance of this cadmium-containing protein is not yet clearly known, but it points to the possibility that the mineral functions in some basic biological system. Metallo-thionein containes 2.9 per cent cadmium, 0.6 per cent zinc and 4.1 per cent sulphur. Because of its high metal and sulphur content, the protein complex has been termed metallothionein. The high sulphur content in metallothionein is probably due to a large number of cysteine residues providing SH groups for binding the cadmium and zinc.

Chromium

Chromium occurs in all plant and animal tissue, in traces. Twenty parts of chromium are present in one billion parts of blood. Certain cell proteins can have concentrations of chromium much higher. The higher concentration of the metal in cells has led to studies of chromium which indicate a probable role it plays together with insulin in glucose metabolism. In animals made chromium-deficient by deprivation, fasting blood sugar levels are elevated followed by glycosuria. The process can be reversed by supplementing diet with chromium. Recent studies in man have shown the ability of chromium to raise abnormally low fasting blood sugar levels and to improve faulty uptake of sugar by body tissues. Infants suffering from severe malnutrition and inability to use sugar have been reported to make rapid recovery when small amounts of chromium are added to their diet.

The average daily human diet contains about 80 to 100 µg of chromium of which only 2 to 5 µg is absorbed. The absorbed chromium is stored in the tissues, from which it is released when glucose is ingested. The tissue levels of chromium vary widely as measured from different sites and at different times. Further studies may reveal the role of chromium in metabolism and its nutritional significance. It may be

TABLE 6.20

Mineral	Metabolism	Physiological Function	Clinical Application	Requirement	Food Source
1. Iron (Fe)	Absorption according to body need controlled by mucosal block. Transferrin mechanism: aided by Vit. C, gastric HCl. Transport-transferrin storage ferritin, haemosiderin excretion from tissue in minute quantities, body conserves and reuss.	Haemoglobin formation, cellular oxidation (Cytochrome system producing ATP).	Growth (milk anaemia). Pregnancy demands, Deficiency anaemia, Excess-haemo-Siderosis; haemochromatosis.	Men: 10 mg Women: 18 mg Pregnancy: 18 mg Lactation: 18 mg Children: 10-18 mg	Liver, meats egg yolk, whole grains Dark green vegetables, nuts.
2. Copper (Cu)	Transported bound to an α-globulin as ceruloplasmin, stored in muscle, bone, liver, heart, kidney and central nervous system.	Associated with iron in: enzyme systems. Haemoglobin synthesis absorption and transport of iron. Involved in bone formation and maintenance of brain tissue and myclin sheath n nervous system.	Hypocupremia: Nephrosis, Malabsorption Wilson's disease, excess copper storage.	2–2.5 mg. Diet provides 2 to 5 mg	Liver, meats seafood: whole grains nuts.
3. Iodine (I)	Absorbed as iodides, taken up by thyroid gland under control of thyroid-stimulating (TSH). Excretion by kidney.	Synthesis of thyroxine the thyroid hormone regulating cell oxidation.	Deficiency endemic colloid goitre, cretinism.	Men: 140 µg Women:100 µg Infants: 25 to 45 µg Children: 55 to 140 µg	Iodized salt, sea foods.
4. Manganese (Mn)	Absorption limited. Excretion mainly by intestin.	Activates reactions in: urea formation protein metabolism, glucose oxidation, lipoprotein clearance and synthesis of fatty acids.	No clinical deficiency observed in humans, Inhalation toxicity in miners	Unknown diet provides 3 to 9 µg	Cereals, soyabeans, nuts, tea, coffee.

5. Cobalt (Co)	Absorbed chiefly as constituent of Vit. B_{12}	Constituent of Vit. B_{12}. Essential factor in red blood cell formation.	Deficiency associated with Vit. B_{12} deficiency pernicious anaemia	Unknown	from performed Vit. B_{12}
6. Zinc (Zn)	Transported with plasma proteins Excretion largely intestinal stored in liver muscle, bone and organs.	Essential enzyme constituent. Carbonic anhydrase, carboxy peptidase. Lactic dehydrogenase combines with insulin for storage of the hormone.	Possible relation to liver disease	Unknown average diet provides 10–15 mg	Widely distributed, liver, seafood.
7. Molybdenum (Mo)	Minute traces in the body.	Constituent of specific enzymes involved in: Purine conversion to uric acid. Aldehyde-oxidation.	—	Unknown	Organ meats, milk, whole grains. leafy vegetables.
8. Fluorine (Fl)	Deposited in bones and teeth Excreted in urine	Associated with dental health.	Small amounts Prevent dental caries. Excess causes endemic dental fluorosis.	—	Water (1 p.p.m. FL)
9. Selenium (Se)	—	Associated with fat metabolism.	Constituent of "factor 3" which acts with Vit. E to prevent fatty liver	—	—
10. Chromium (Cr)	—	associated with glucose metabolism.	Infants unable to metabolize sugar and adult diabetics show definite improvement when small amounts of chromium added to diet. Possible link with cardiovascular disorders and diabetes.	—	—

worthwhile to speculate the possible link with chronic disease processes such as cardiovascular disorders and diabetes.

Summary of the trace minerals is given in Table 6.20.

Enzymes and Inorganic Elements

Inorganic elements are required in small amounts for the activity of many enzymes. The metal probably acts by forming a bond between the enzyme-protein groups, and the substrate. The metal requirements of several enzymes are listed below in Table 6.21.

TABLE 6.21

Enzyme	Reaction	Meta
1. Carbonic anhydrase	$CO_2 + H_2O \rightleftarrows H_2CO_3$	Zn
2. Inorganic pyrophosphatase	Pyrophosphate $+ H_2O \rightarrow PO_4$	Mg
3. Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$	Fe
4. Cytochromes	Electron transport	Fe
5. Tyrosinase	Tyrosine $+ \frac{1}{2}H_2O \rightarrow$ Hallochrome	Cu
6. Lactase	Phenols $\rightarrow$ ortho and paraquinones	Cu
7. Ascorbic acid oxidase	Ascorbic acid $\rightarrow$ Dehydroascorbic acid	Cu
8. Prolidase	Glycylproline $\rightarrow$ Proline	Mn
9. Carboxypeptidase	Chloroacetyl-tyrosine $\rightarrow$ Tyrosine	Mg
10. Glycylglycine dipeptidase	Glycylglycine $\rightarrow$ Glycine	Zn

From McElroy and Swanson, *Scientific American*, January 1953, p 22.

Further Reading

B. Harrow and A. Mazur, *Text Book of Biochemistry* (New York: Saunders, 1958).

C.L. Comar and F. Bronner, *Mineral Metabolism* (London: Academic Press, 1962).

E.J. Underwood, *Trace Elements in Human and Animal Nutrition* (London: Academic Press, 1962).

S.R. Williams, *Nutrition and Diet Therapy* (London: Mosby Company, 1973).

H.A. Harper, *Review of Physiological Chemistry* (London: Lange, 1969).

E.S. West, *et al.*, *Text Book of Biochemistry*. (Delhi: Amerind, 1974).

Intermediary Metabolism

Introduction

The most striking feature of the chemistry of the living cell is the dynamic state of its constituent molecules. Cells contain large number of enzymes that catalyse the formation and breakdown of molecules. Once in the blood stream, absorbed nutrients are distributed to the cells of the body, where they undergo many remarkable changes. The sum total of these changes brought about by the chemical tools called the enzymes, has been named metabolism. The metabolism not only denotes (1) the energy-requiring synthesis of new complex organic compounds similar to those previously digested but also includes (2) the energy-releasing degradation of absorbed nutrients to such simple end-products as carbon dioxide and water.

The first aspect of metabolism of tissue formation is commonly referred to as 'anabolism' and the metabolism of tissue breakdown is known as 'catabolism'. The terms dissimilation or catabolism denote the total breakdown process undergone by foodstuff down to the final end products. The end products in the case of carbohydrates and fats are CO_2 and H_2O; with amino acids, the end-products containing nitrogen in the form of urea, also appear.

The opposite transformation—the building up of storage, structural, and functional materials from simple food stuffs or intermediates is termed assimilation or anabolism. Catabolic processes liberate energy as the energy content of a complex food stuff is greater than that of its simpler degradation products. On the contrary the anabolic processes will proceed only when energy from elsewhere is put into them. Anabolism and catabolism are the composing processes of reversible chemical reactions:

$$\text{Small molecules} \xrightarrow{\text{anabolism}} \xleftarrow{\text{catabolism}} \text{large molecules}$$
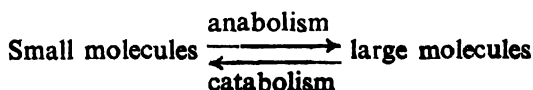
Growth of the organism takes place as in the period of immaturity when the processes of tissue synthesis, anabolism, exceed those of tissue breakdown, catabolism. There is no change in tissue mass as in the period of normal maturity, when the processes of anabolism and catabolism balance. In old age the rate of tissue catabolism exceeds that of anabolism with the decline in tissue mass.

The complex molecular structures of food stuffs are not changed into simpler discard products by a single chemical reaction during the course of their oxidative breakdown in the body but proceed through a series of well-defined sequence of chemical reactions. The foodstuff is metabolized along a specific pathway which indicates the sequence of intermediate chemical structures through which the foodstuff passes until the final waste products are formed. There may be a large number of intermediate stages for what may appear to be a relatively simple change and each stage may be catalysed by its own highly specific enzyme. Intermediary metabolism is largely concerned with the investigation of the way in which particular compounds are broken down in the living cell and the steps by which others are built up. It aims to know the changes undergone by substances during the process of their utilization by the body and of the reactions in which they take part after their absorption.

When the sequence of the chemical reactions taking place normally in a particular tissue, is known, the disordered metabolism in that tissue may be pinpointed for treatment in a logical and systematic manner, perhaps by administering substances which are lacking or by drugs which slow down the production of harmful substances. There are a variety of dietary proteins, carbohydrates, and fats to be metabolized and it may appear to be beyond the capacity of a cell to participate in the large number of chemical changes involved in the process of metabolism. The problem is considerably simplified by intermediary metabolism and the number of steps required to release the available energy from the large number of substrates is very small. The intermediary metabolism proceeds in three major phases:

Phase I
This involves the intestinal digestion and absorption and similar process in tissue when storage material is mobilized for dissimilation or catabolism. In Phase I the polysaccharides are transformed into simple hexase sugars; fats into glycerol and fatty acids and proteins into amino acids. From a variety of complex molecules of foodstuffs, a few small soluble molecules are obtained such as glucose and several closely related isomers; glycerol and about ten fatty acids of varying chain length; and about twenty dissimilar amino acids. Relatively little energy is liberated in the hydrolytic reactions of Phase I. The reactions appear to prepare foodstuffs for metabolism proper.

Phase II

Phase I products are partially oxidized in Phase II along converging pathways with the formation of CO_2, H_2O. nitrogenous waste products, and one of the three acids—acetic acid (active acetate form), α-Ketoglutaric acid, and oxaloacetic acid.

All the carbon atoms of the fatty acids, two-thirds of the carbon atoms of carbohydrate and glycerol, and abou: half the amino acids give rise to acetic acid. The two keto acids are derived from other amino acids. About one-third of the available energy of complete dissimilation or catabolism is released during Phase II.

Phase III

The three acids derived from Phase II metabolism, undergo complete oxidation in Phase III through a complicated metabolic cyclical pathway in the cells called the 'citric acid cycle' as citric acid is the chief intermediary product. The acids are oxidized to CO_2 and H_2O and the remaining two-thirds of the available chemical energy is released.

Phase III comprises the final metabolic pathway common to the carbon compounds of all major foodstuffs providing a set of related and interchangeable intermediates through which the major metabolic products can be transformed one into the other. Phase II and Phase III metabolism take place in all cells which require supply of oxygen with minor differences in enzymes depending on species.

The metabolic process reveals an extensive network of reactions in which one reaction sequence is repeatedly linked to another through the chemical intermediates that are common to each one. A dozen sequences, for example, are linked through the common metabolite, pyruvate: some of them are degradative reactions giving rise to pyruvate whilst in other cases the compound initiates a pathway of biosynthesis. The reaction network provides a means by which pyruvate can be rapidly synthesized and degraded by enzymes. The concentrations of these metabolites within the cell remain at any moment of time nearly constant, not because the molecules are chemically stable but because a steady state is reached with a balance maintained between the rates at which particular molecules are broken down and reformed. A continual supply of energy and material is required by the cell in order to maintain the steady state. The functional operations of tissues such as muscular contraction, the propagation of nerve impulses, the secretory work of various glands, the selective absorption processes of the intestine, and the excretory processes of the kidney and other organs require a multitude of chemical reactions to provide necessary energy and specific chemical substances.

The chemical processes primarily concerned in muscle contraction are those which yield energy in a form that can be transferred to the contractile elements and operate them. Adenosine triphosphate (ATP) is often one of the products formed by enzyme systems that release energy in these chemical processes involving chiefly the oxidative breakdown of

sugars and fatty acids. This is highly reactive, or energy-rich compound which transmits its phosphate bond energy to the muscle fibrils and causes contraction.

The breakdown and oxidation of glucose by the same pathways as in muscle metabolism, provide largely the energy needed for the transmission of impulses in the central nervous system and nerves. ATP is formed which provides the free energy necessary for the synthesis of acetylcholine and for other processes involved in the generation and transmission of action currents.

The pituitary, thyroid, parathyroid, pancreas, and the sex glands are endocrine glands secreting the chemical messenger hormones. The hormones perform specific roles in regulating tissue metabolism. Iodine and tyrosine (amino acid) combine in the cells of the thyroid gland to form diiodotyrosine—the precursor of the hormone thryroxine. The reactions within the endocrine glands provide the necessary precursors as well as the energy required for synthesis.

Numerous chemical reactions take place in the cells of intestinal mucosa providing energy for the active transport of some sugars and other substances from the lumen into the blood. Glycerides and phospholipids are formed and then pass into the lymph and blood stream. Enzymes of the intestinal cells break down sucrose and other substances during the process of digestion.

Glucose, sodium and many other substances are reabsorbed from the glomerular filtrate by the epithelial cells of the kidney tubules. This is performed by active transport through energy-yielding chemical reactions. The proper distribution and balance of ions and diffusible molecules between the extracellular and intracellular fluids are in fact due to the utilization of a considerable proportion of energy production in active transport across the membranes throughout the body.

Metabolism is therefore not only concerned with the chemistry of tissue formation and breakdown but also with the chemical processes in tissues necessary for the formation of various specific compounds involved in the operation and regulation of the metabolic machine and in providing the energy. Energy-producing reactions give rise to substances to be utilized for synthesis, the intermediate stages providing the energy for this. The cells carry out such reactions at approximate neutrality, at body temperature, and at high speed with the aid of enzymes. For the hydrolysis or the oxidation of substances like fats, proteins in the laboratory, strong acids or alkalies or oxidizing agents and high temperatures are needed. Such reagents and temperatures are incompatible with the existence of living matter. It takes 10,000,000 times as great a concentration of hydrogen ion as of the enzyme sucrase to decompose a given amount of cane sugar in a given time at body temperature.

The study of metabolism is interrelated with that of the mode of action of specific enzymes and thus with the chemistry of life itself. Many common pathways are followed in general metabolic processes of all

organisms—animals or plants. The chemical process involved in the metabolism of glucose by yeast is different from that occurring in the tissues of higher animals only in a few details. Many reactions taking place in microorganisms have been found to occur in animal metabolism.

Derangements of certain phases of human metabolism often give rise to specific pathological conditions. Diabetes is produced due to the failure of pancreas to secrete insulin result..ng in a marked decrease in the metabolic reactions for the breakdown of glucose and accelerated degradation of fats and tissue proteins. A generalized decrease in the oxidation of foodstuffs in all the tissues of the body occurs when the thyroid gland fails to produce thyroxine with a simultaneous failure in the synthesis of tissue structures. Calcium and phosphorous metabolism are deranged markedly as a result of the failure of the parathyroid glands to form their hormone. Thiamine - deficiency in diet causes a deficiency of carboxylase and other enzymes required for the oxidation of pyruvic acid formed in glucose metabolism. Pyruvic acid accumulates in tissues and blood giving rise to beriberi. Many biological oxidations require flavoproteins which fails to be synthesized due to Riboflavin deficiency. Coenzymes I and II are not formed adequately in niacin deficiency. These coenzymes are also concerned as components of biological oxidation chains. Lack of sufficient oxygen supply to tissues prevents adequate tissue synthesis due to the failure of the energy supply with the result that the tissue-breakdown exceeds the rate of its formation. Death follows quickly on acute oxygen deprivation, by interrupting the chemical process for the supply of energy to the brain.

Drugs, vitamins, hormones are used for altering one or more metabolic processes of animal tissues or of invading organisms through the enzyme systems of the cells. Metabolic reactions of the nervous system are slowed down by narcotics •like barbiturates through the inhibition of the dehydrogenase enzyme systems. The invading organisms are successfully treated with sulpha drugs which interfere with their metabolic reactions by inhibiting certain enzymes of the organisms. The toxic effects of poisons are produced by interference with the metabolic processes. Cyanide is a deadly poison. This is due to the fact that the cyanide combines with cytochrome oxidase making it inactive to a large proportion of cellular oxidations. The sulphydryl groups (− SH) of enzymes combine with the mercuric ions with the resultant inactivity. All these indicate the importance of the study of metabolism in understanding its normal process as well as its abnormal states and the solution thereof.

Synthesis of Adenosine Triphosphate (ATP)

All chemical reactions involve energy exchanges. The energy values are generally expressed in heat units—the calories.

A chemical reaction which liberates heat is known as exothermic

and the one which takes in heat is endothermic. The energy which can be made available for performing useful work by a chemical reaction is not however always equivalent to the heat liberated. In metabolic processes, the available energy, which is metabolically usable, is, of importance. Reactions which release usable energy are called exergonic and those which require external energy to be supplied are endergonic. Most catabolic (dissimilation) reactions are exergonic.

One mole of glucose (180 g) on oxidation under ideal condition liberates energy equivalent to 686,000 calories. If this amount of heat were to be released instantaneously, the oxidation of a few molecules of glucose could destroy a living cell. Mammalian cells operate at a constant temperature. Heat must be conducted from one temperature to another in order to do work. This does not happen in living cells and hence a different form of energy must be used. Adenosine triphosphate (ATP) provides a central store of energy in animal tissues and the problem of the utilization of food materials by oxidation is largely the problem of how this oxidation results in ATP synthesis. The energy content of glucose is 686,000 calories per mole above the energy content of its oxidation products (CO_2 and H_2O). The reverse of the reaction is the synthesis of 1 mole of glucose from CO_2 and H_2O as happening in plants during photosynthesis, where energy equivalent to 686,000 calories must be supplied to the system from an outside source.

Loss of an electron is oxidation and its acquisition is reduction. The definition can be extended to include in oxidation the loss of an atom or group which has a weak affinity for electrons. Hydrogen is such an atom. The addition of such an atom or group to a molecule constitutes reduction. Oxidation also denotes the acquisition of a group or atom such as oxygen, having a high affinity for electrons. Loss of such an atom is reduction. There are three oxidation reactions which are of importance in biochemistry as indicated:

1. The loss of electron
2. The loss of hydrogen
3. The acquisition of oxygen

A complex molecule has a higher energy content than the atoms or simpler molecules from which it is built, because of the energy of formation of the chemical bonds holding it together. As the bonds are broken, this bond energy is liberated. The structure of the whole molecule determines the bond-energy of the link between two given atoms of a molecule. The organic phosphates constitute an important group of compounds with the general formula, $R.O.PO(OH)_2$, 'R' being the organic radical derived from glucose, creatine, etc. Most organic phosphates on hydrolysis liberate phosphoric acid and energy equivalent to 2,000 to 3,000 calories per mole, is made available as heat. The substance in this case, is referred to as low-energy phosphate compound; it is represented by the formula $R - ph$, ph being the phosphate group. Some organic phosphates of special structural types when hydrolysed, energy equivalent to 10,000 to

12,000 calories is made available. These are high-energy phosphate compounds and are designated as R ~ ph.

Low-energy phosphate compounds are glucose-1-phosphate and glucose-6-phosphate; fructose diphosphate; phosphoglyceraldehyde; monophosphoglyceric acid; adenosine monophosphate (AMP). High-energy phosphate compounds include adenosine diphosphate (ADP); adenosine triphosphate (ATP); creatine phosphate; diphosphoglyceric acid; phosphopyruvic acid; acetyl phosphate.
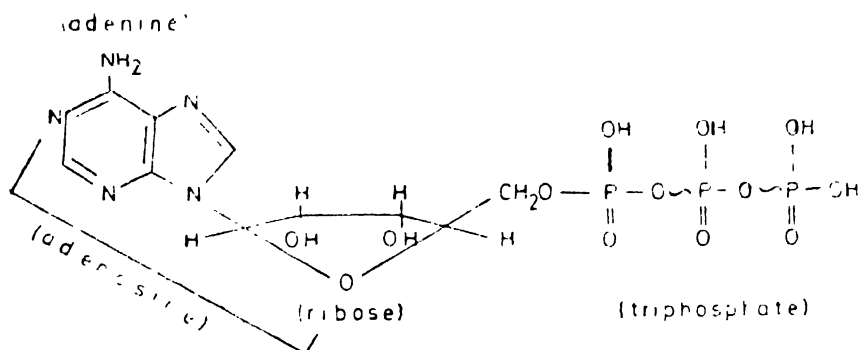
Adenosine triphosphate (ATP)

The high-energy phosphates play important role in metabolism. The phosphate radical can be transferred directly to another organic molecule without much of the high energy of the reactant being lost as heat. The product is a phosphorylated compound which may or may not retain a high-energy phosphate bond but its total energy content would be higher than that of the non-phosphorylated compound by 5,000 to 12,000 calories per mole. Interaction of ATP with glucose are examples:

Glucose + adenosine − ph ~ ph ~ ph
           (ATP)
              →   glucose − ph   + adenosine − ph ~ ph
                  (glucose-6-phosphate)   (ADP)

with creatine:
    creatine + adenosine − ph ~ ph ~ ph
              ⇄   creatine ~ ph   + adenosine − ph ~ ph
                  (creatine phosphate)   (ADP)
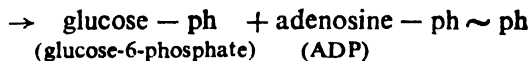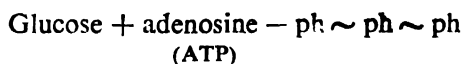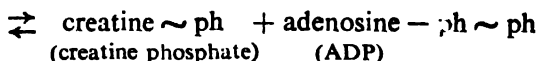
The energy of dissimilation (catabolism), is utilized to synthesize high-energy phosphate compounds, such as ATP instead of being lost immediately as heat. These compounds are stored and the energy content in them is utilized as required.

It may be that the low-energy phosphate compounds are converted into high-energy phosphate derivatives, the phosphate groups are used to phosphorylate ADP producing ATP. The energy of dissimilation is

transferred to the products of reaction. The oxidation of phosphoglyceral-
dehyde to phosphoglyceric acid in the body takes place:

$$ph-G.CHO \quad\quad +HO \enspace ph \rightarrow \quad\quad ph-G.CH \Big\langle {}^{OH}_{O-ph}$$

(phosphoglyceraldehyde)(phosphoric acid)   oxidatior
                                                                    (-2H)

$$ph-G.COOH \quad\quad +ADP \quad\quad ph-G.CO.O-ph$$

(phosphoglyceric acid)   (diphosphoglyceric acid)
  +ATP

The sole source of energy that cells can use directly comes from the
high-energy phosphate. It is used: (1) to effect chemical synthesis; (2) to
perform work such as muscular, osmotic, secretory; (3) to liberate heat by
hydrolysis maintaining the body temperature but dissipating the metabolic
energy.

High-energy esters: Coenzyme A

Besides the special phosphates, the acyl derivatives $(R-CO-)$ of mercap-
tans $(HS-R')$ constitute another group of metabolic intermediates of
high-energy type, e.g., thio-esters, $R-CO-S-R'$. The thio-esters on

Structure of CoA

hydrolysis yield the acid R—COOH and the mercaptan, HS—R' with the release of as large an energy as 8,000 to 10,000 calories per gram mol in contrast to 2,000 calories released from the hydrolysis of oxygen esters. R—CO—O—R'. The high-energy thio-esters like that of phosphates, are represented as R—CO∼S—R'. The acyl derivative of coenzyme A is the most important metabolic intermediate in this group. This coenzyme is widely distributed mercaptan with the chemical structure.

CoA is usually shortened to HS—CoA. Coenzyme A combines with acetate to form 'active acetate'. In the form of acetyl CoA (active acetate), acetic acid participates in a number of important metabolic processes. It is utilized directly by combination with oxaloacetic acid to form citric acid, which initiates the citric acid (Krebs) cycle. Thus acetic acid derived from carbohydrates, fats, or many of the aminoacids undergoes further metabolic breakdown via this final common pathway in metabolism. In the form of active acetate, acetic acid also combines with choline to form acetylcholine, or with the sulphonamide drugs which are acetylated prior to excretion.

Pyruvic acid, $CH_3.CO.COOH$, has a higher energy content than acetic acid, CH_3COOH. Oxidation of pyruvate metabolically to acetate gives rise to energy which is not wasted as heat. The actual product is the coenzyme A ester of acetic acid (acetyl-CoA, $CH_3—CO∼S—CoA$). The energy released from the pyruvate is mopped up in the formation of this high-energy compound.

The product of decarboxylation of α-ketoglutarate in the citric acid cycle is a coenzyme A derivative called active succinate (Succinyl-CoA). Active succinate and glycine are involved in the first step leading to the biosynthesis of haem.

A major function of pantothenic acid is its role as a constituent of coenzyme A. The total pantothenic acid content of the cell cannot be accounted for as CoA. A significant amount of the cellular pantothenic acid is protein-bound, acyl carrier protein (ACP).* The acyl carrier protein is a coenzyme required in the biosynthesis of fatty acids and CoA is primarily concerned with the catabolism of fatty acids excepting its role in cholesterol biosynthesis and thus in steroid hormones.

The production of acetyl-CoA, or any acyl-CoA compound is tantamount to the production of a molecule of ATP from ADP, at least as far as biological energetics is concerned:

$$CH_3.CO∼S.CoA + ADP + HO—ph$$
$$\rightleftarrows \ CH_3.COOH + HS.CoA + ATP.$$

Mechanisms of Biological Oxidation

The catalytic acitivity of enzyme systems determines all the reactions of

*B. L. Pugh and S. J. Wakil, *J. Biol. Chem.*, 240 (1963), p. 4727.

metabolism. Some soluble proteins, enzyme-protein or apoenzyme, together with various accessory substances constitute these systems. Mg^{++}, or PO_4^{---} ions are called cofactor which accelerate the enzyme action. Coenzymes are complex organic but non-protein substances acting as essential intermediate carrier of products of enzyme-catalysed reactions. The enzyme-protein is generally specific for a particular chemical reaction or type of reaction but a particular coenzyme is less specific and may act as a carrier in a number of different systems. The coenzyme as a matter of fact, is one of the reacting substrates being transformed chemically into some other product. The product can regenerate the coenzyme for a repitition of the process. The over-all reaction is $A-B+C \rightarrow A-C+B$. The coenzyme functions as below:.
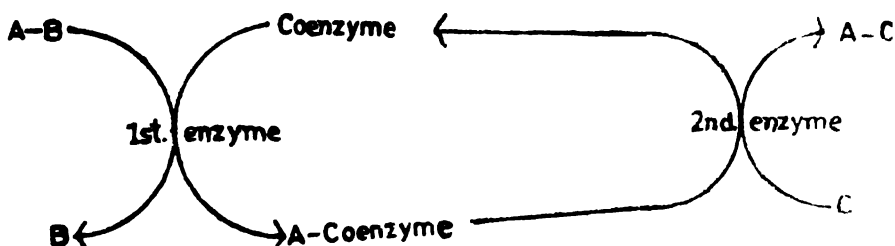
There are certain conjugated proteins where the enzyme-protein is chemically combined with an organic non-protein unit called the prosthetic group of the protein. The prosthetic group acts as an intermediate carrier of products of the reaction, the enzyme having a built-in coenzyme, such as the flavoproteins and cytochrome oxidase.

DEHYDROGENATION

Many forms of tissue oxidation do not require addition of atmospheric oxygen to the substrate but addition of water or related substance, $R-O-H$ followed by dehydrogenation or removal of hydrogen. A specific dehydrogenase enzyme catalyses the transfer of 2H to the appropriate coenzyme acceptor which undergoes reduction. These types of oxidation are anaerobic where a supply of coenzyme is needed but not oxygen. The regeneration of the coenzyme from the reduced form in the final phase however depends on oxygen.

The coenzyme acceptor for dehydrogenase reactions is commonly a complex derivative of nicotinamide called nicotinamide adeninedinucleotide, NAD or CoI or DPN. NAD is however the more appropriate name. In its reduced form, NAD. 2H, the hydrogen is attached to the nicotinamide portion of the molecule.

Some of the dehydrogenase enzymes need phosphate derivative of NAD, designated as NADP or coenzyme II or TPN as their hydrogen acceptor. Nicotinamide belongs to the vitamin B group and must be provided in

(nicotinamide)

Nicotinamide adeninedinucleotide (NAD)
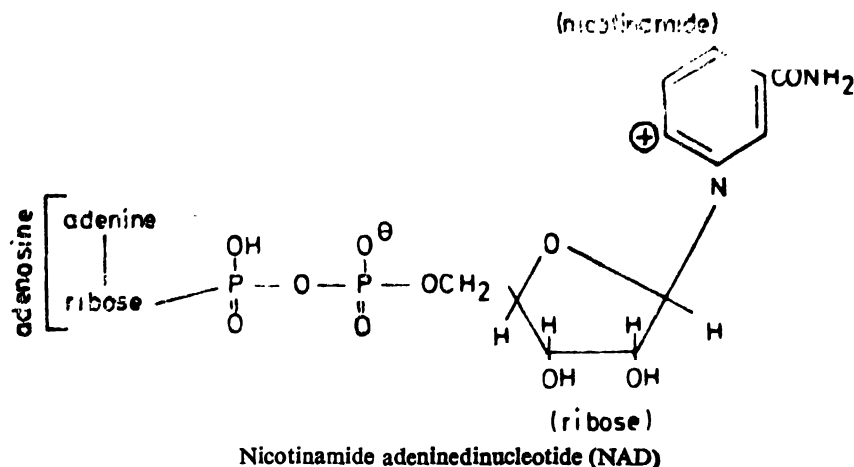
diet. The reduced coenzyme passes its hydrogen on reoxidation to hydrogen carriers ultimately forming water with molecular oxygen.

TYPES OF OXIDATIVE REACTION

Biological dehydrogenation can be indicated by the following:

1. Oxidation of lactic acid to pyruvic acid involves NAD as the hydrogen carrier. The reaction is fully reversible without liberation of energy provided some alternative process does not lead to the reoxidation of the reduced coenzyme, NAD.2H.

2. Oxidation of phosphoglyceraldehyde to phosphoglyceric acid provides an important energy-liberating step in the pathway of glucose metabolism:

$$ph-G-CHO + HO-ph + ADP + NAD \rightarrow$$
$$ph - G - COOH + ATP + NAD.2H.$$
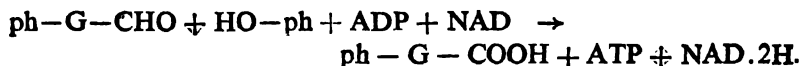
This is the classical example of energy-trap by ATP formation. The oxidation of each molecule of the aldehyde to acid liberates usable energy—ADP giving rise to one ATP and one hydrogenated NAD molecule (NAD.2H). Each NAD.2H molecule generates 3 ATP molecules as it is reconverted into the coenzyme NAD by oxygen and the appropriate enzyme. The reaction proceeds till the coenzyme is completely reduced in absence of oxygen, unless an alternative hydrogen acceptor is available. The alternative can be pyruvic acid as in muscle.

3. The oxidation of the keto-acid, pyruvic acid ($CH_3 CO.COOH$) to CO_2 and acetic acid (CH_3COOH) by decarboxylation is an important energy-liberating stage. Coenzyme is reduced and the acetic acid appears as its high-energy ester with coenzyme A,

$$CH_3.CO.COOH + NAD + HS.CoA \rightarrow$$
$$CH_3.CO \sim S.CoA + NAD.2H + CO_2$$
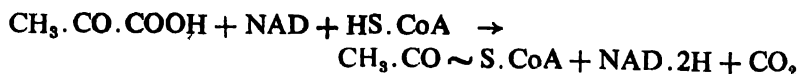
The conversion of pyruvate to acetate is irreversible—one molecule of pyruvate yielding one high-energy acetyl-CoA alongwith one reduced NAD. Other ketoacids like ketoglutaric acid, undergo similar oxidation.

The dehydrogenases liberate hydrogen to be accepted by NAD or NADP or a flavin. The hydrogen remains attached to the coenzyme for a short while and then transferred elsewhere for the regeneration of the coenzyme. The hydrogen load is passed from one to another through the mediation of a bucket-chain of carrier substances, the links in the chain being enzymes with prosthetic groups. Finally it reacts with oxygen to form water :

$$NAD.2H + \tfrac{1}{2}O_2 \xrightarrow{\text{many steps}} NAD.H_2O.$$
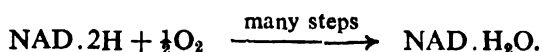
Metabolic energy equivalent to three ATP molecules is generated as one molecule of reduced coenzyme is oxidized through the respiratory chain. It is not known as to how the oxidative phosphorylation is coupled with hydrogen and electron-transport mechanism.

The flavoproteins, the metal-containing enzymes, catalyse the transfer of hydrogen from reduced-coenzymes to the prosthetic group of the enzyme. The prosthetic group is a yellow pigment called flavin or flavin-adenine dinucleotide, FADN, which is a derivative of riboflavin, vitamin B_2. This indicates another metabolic function of vitamins B group.

Cytochrome pigments are conjugated proteins with iron-porphyrin prosthetic groups. The proteins are not globin and the prosthetic groups are similar to but not identical with the haem of haemoglobin. The cytochrome carriers in the cells are alternately reduced and oxidized in appropriate order. The first cytochrome undergoes reduction from ferric iron to ferrous iron by reduced flavins, which then reduces the next cytochrome and itself is converted into the oxidized state. The final reduction is of cytochrome oxidase, which catalyses the reduction of its own iron-porphyrin prosthetic group by reduction cytochrome, regenerating the oxidized cytochrome.

Unlike other carriers of the respiratory chain, reduced cytochrome oxidase is readily oxidized by molecular oxygen. The hydrogen from the substrate combines with respiratory oxygen to form water.

Addition of 2H atoms is involved in the reduction of coenzymes and flavins. The iron-containing cytochromes are however reduced by electron transport involving change of ionic charge.

The dehydrogenation reaction, involving removal of hydrogen atom, undergoes acidic ionization to produce a proton, which is positively charged and denoted by H^+, and an equally but oppositely charged, electron, denoted by ε'.

$$H \rightleftharpoons H^+ + \varepsilon'$$
$$\text{(atom)} \quad \text{(proton)} \quad \text{(electron)}$$

Oxidation is not only gain in oxygen or loss of hydrogen but also loss of electrons. Reduction denotes gain of electrons.

$$Fe^{++} \underset{+\varepsilon'}{\overset{-\varepsilon'}{\rightleftharpoons}} Fe^{+++}$$
$$\text{(reduced)} \qquad \text{(oxidized)}$$
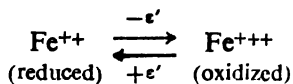
Iron-containing cytochrome (Cyto—Fe^{+++}) and cytochrome oxidase (CyO—Fe^{+++}) behave in the same manner.

$$H \text{ (from substrate through Co and flavin)} \rightarrow H^+ + \varepsilon'$$
$$Cyto - Fe^{+++} + \varepsilon' \rightarrow Cyto - Fe^{++}$$
$$Cyto - Fe^{++} + CyOx - Fe^{+++} \rightarrow Cyto - Fe^{+++} + CyOx - Fe^{++}$$
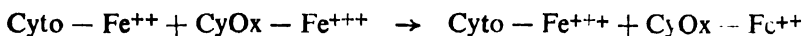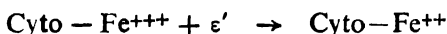
Reduced cytochrome Oxidase, CyOx — Fe^{++}, transfers an electron to molecular oxygen. This is an unique property of the reduced cytochrome oxidase:

$$2CyOx - Fe^{++} + \tfrac{1}{2}O_2 \rightarrow 2CyOX - Fe^{+++} + O''$$
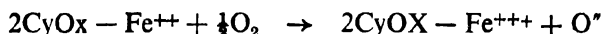
Protons from the initial ionization then react with the negatively charged oxygen to form water:

$$2H^+ + O'' \rightarrow H_2O$$
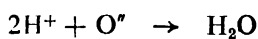
The haem of haemoglobin closely resembles the porphyrin groups of cytochromes and cytochrome oxidase and yet the proteins derived from them differ fundamentally. Haemoglobin combines with oxygen reversibly but its iron is not oxidized. The iron of cytochromes however is oxidized and reduced reversibly, but the iron is not direct involved the exception being the reduced cytochrome oxidase acting as oxidizing agent.

CELLULAR ORGANIZATION OF RESPIRATION : ELECTRON TRANSPORT PARTICLE

The aerobic respiratory processes of the cell are conducted by mitochondria only. An organized repeating unit occurs in the mitochondria for both electron transfer and oxydative phosphorylation. This unit has been purified as discrete particle, the electron transport particle, ETP. All the components of the respiratory chain in determined molecular proportions and in organized spatial sequence have been found to be present in the ETP. The hydrogen from reduced coenzymes is accepted by the ETP which uses molecular oxygen as terminal acceptor of hydrogen without the involvement of any other enzyme or factor. Slight damage of the ETP causes the aerobic oxidation sequence to proceed but the simultaneous generation of high-energy phosphate compounds is adversely affected. Only portions of the electron transport sequence are possible when greater damage to the ETP causes its fragmentation which still retains its entity.

BIOLOGICAL OXIDATION AND ENERGY RELEASE RELATIONSHIP

Generation of high-energy compounds occurs in two distinct types of oxidative energy release:

1. Anaerobic oxidation takes place at the substrate level. The oxidation of phospho-glyceraldehyde generating ATP or the oxidation of pyruvate generating acetyl-CoA.

2. Each molecule of reduced coenzyme forms three molecules of ATP from three molecules of ADP alongwith phosphate in aerobic oxidation through the respiratory chain. This is known as electron transport phosphorylation or respiratory phosphorylation.

The study of intermediary metabolism is concerned with the under-standing of the changes undergone by substances in the process of their utilization by the body and of the reactions in which they take part after their absorption. Biochemistry deals with the processes and reactions by which compounds are broken down in the living cells and others are built up.

Methods of Study of Metabolism

Development of laboratory methods and instruments has made remarkable progress in the study of metabolism. Procedures such as paper, column, and gas chromatography, counter-current extraction and ultra-centrifuga-tion have enabled the isolation and purification of a number of biological substances from small amounts of tissues and fluids Electrophoresis and ultracentrifugation have afforded the means to establish the purity and physicochemical properties of enzymes and other proteins. Minute amounts of biological substances have been qualitatively and quantitatively estimated using such methods as characteristic light absorption in the ultraviolet, visible, and infra red regions of the spectrum, measured by appropriate colorimeters, spectrophotometers, and fluorimeters. The biological reaction mechanisms are understood by the characteristic behaviour of free radicals in powerful magnetic field—the electron spin resohance (ESR).

The use of radioactive isotopes as tracers has made great strides in the understanding of metabolic reactions. The study of tissues, cells, and cell components with the help of ultraviolet, phase, and electron microscopy alongwith histochemical techniques, has revealed the localization and quantitative relations of the enzymes and other substances.

Analysis of urine may provide information about intermediary metabolism. The amount of urea in urine is increased with increase in the protein content in diet. The urea excretion is diminished when the protein intake is decreased. This indicates that urea is one of the end-products of protein metabolism. It has to be remembered in this connection as to whether protein gives rise to end-products other than

urea as well as whether urea is derived from substances other than proteins. Also the compounds found in urine may not be the normal intermediates but those produced by the emergency mechanism.

Useful information may be obtained by feeding possible intermediates. Metabolic studies can be carried out utilizing material from several levels of biological organization. The intact animal in which all cells of all tissues are intact operating in an integrated manner, constitutes the highest level.

Isolated organs, such as liver, constitute the second level. If an organ is removed and maintained at body temperature in suitable solution, it remains alive and can carry out the chemical reactions as accomplished in the intact body. When the solution is perfused through the blood vessels of the isolated organ, examination of the perfusate can provide information about the intermediate breakdown products.

Thin slices of an organ with most of the cells and membranes intact, provide the third level of organization. The normal spatial arrangement of the enzymes and other constituents in the cell is preserved in the slice method.

Mechanical grinding, sonic waves, etc., destory the cell membranes, cellular organization and compartmentation. This method of study constitutes the fourth level of organization. The finely minced tissue is known as homogenate and the breakdown products can be identified and estimated in the incubation mixture. This method provides a means to study the enzyme activity as well as the means for the separation of the tissue into cytoplasm, nuclei, microsomes, and mitochondria by differential centrifugation for the study of the metabolic properties of the cellular components.

The fifth and the lowest level of organization in metabolic study is the enzyme in solution either in the form of a crude extract or in a highly purified homogeneous state.

Useful metabolic information is obtained by experiments on each of these preparations and the correlation of data obtained at all levels of organization helps in the understanding of the integrated metabolism of the animal as a whole. One of the methods may be adequate for a given problem, while in others a combination of procedures is necessary.

Intermediary metabolism has been investigated using a number of surgical methods. The part played by an organ in metabolism can be studied from the isolated organ or by the interferance with its blood supply. In the technique of angiostomy by inserting cannulae leading to the blood vessels, blood may be withdrawn or material injected at any time. Insertion of cannulae in the hepatic, renal or portal veins or a communication made between the portal vein and the inferior vena cava to short-circuit the liver so that the portal blood from the small intestine flows direct into the inferior vena cava, as in ECK fistula operation, makes it possible to investigate the role of liver in the metabolism of many substances. A more drastic measure involves the removal of the whole

liver (hepatectomy). Urea formation ceases during the short period that animals remain alive after hepatectomy—indicating the liver to be site of urea formation in the intact animal.

Metabolic processes are disturbed by certain drugs. Liver cells are damaged by carbon tetrachloride, chloroform, or phosphorus. The glycoside phlorizin poisons the renal tubules and prevents glucose reabsorption and thus provides much information about the intermediary metabolism of carbohydrates. The phlorizinized dog excretes glucose in urine after the administration of an amino acid indicating that it is metabolized by way of glucose after deamination.

The role of a substance in metabolism can be determined experimentally by giving a diet deficient in the substance. The amount of nicotinamide nucleotides in the blood and tissues is decreaded when the diet is deficient in nicotinic acid. Information about intermediary metabolism is also provided by some diseases. Diabetes mellitus and carbohydrate metabolism are interlinked.

Metabolic processes are disturbed by genetic defects arising from certain rare hereditary metabolic disorders called inborn errors of metabolism. Gene, a segment of nuclear DNA, controls the cellular enzyme. The enzyme E_2 is deficient or absent when the gene controlling the enzyme E_2 is defective or absent due to heredity abnormality This becomes evident when a substance A is broken down via two intermediates B and C and finally to D. In metabolic disorder products C and D are not formed and substances A and B accumulate in the tissues and may be excreted in the urine. Such disorders include alkaptonuria when tryosine metabolism is defective, phenyl-ketonuria with phenylalanine metabolism impaired.

The breakdown products of food—carbohydrates, proteins and fats, after digestion and absorption, combine with similar compounds in the body to constitute the metabolic pool. The metabolic fate of any particular substance fed to an animal or introduced into a biological system cannot be followed by the usual methods.

Isotopes have been widely used in the elucidation of metabolic processes. Isotopes are elements of same atomic number and hence the same, nuclear charge with different atomic weights. Chemically the isotopes of the same element are identical but they are distinguishable by physical means. They are classified as stable isotopes where the atomic arrangement is stable without any tendency for disintegration, such as ^{12}C and ^{13}C. The radio active isotopes constitute the other class, such as ^{11}C and ^{14}C. The atom here is unstable and disintegrates with emission of α-particles, β-particles (electrons), or α-radiation. Radioactive isotopes which emit α-particles, are not of much importance in biological experimeats. Radioactive isotopes, such as ^{32}P, ^{14}C, and ^{3}H, emit β-particles and are commonly used in biology and medicine so also the radioactive isotopes emitting β-particles and α-radiation, such as ^{131}I. The rate of decay or disintegration differs with different radioactive isotopes. The rate of disintegration of a radioactive isotope is referred to as its half life—that

is the time taken for half of the isotope originally present to disappear. Radioactive phosphorous ^{32}P has a half life of 14.3 days. ^{11}C has a half life of about 20 minutes whereas the half-life of ^{14}C is about 5600 years.

The ideal way for metabolic studies is to substitute into the molecule an atom having the same chemical properties as the atom replaced and possessing properties by which it can be detected in various compounds produced as a result of metabolic reaction by some physical characteristics. The isotopes possess these characteristic and with the development of nuclear chemistry it has been possible to obtain isotopic atoms of practically all of the elements essential to living organisms.

A large number of radioactive and heavy and light isotopes of the elements has been produced in recent years artifically by bombardment of the atomic nuclei with protons ($_1H^1$), deuterons ($_1H^2$), α-particles ($_2He^4$), and neutrons ($_0n^1$). Protons and deuterons contain a single positive charge, two positive charges are present in α-particles while neutrons are without charge. The bombarding particles with the required energy are provided by various devices such as the cyclotron.

The preparation of isotopes and their synthesis into compounds for metabolic and other studies are of special significance in tracing the metabolic pathways The ordinary carbon atoms with atomic weight 12 present in glucose may be replaced by radioactive isotopic carbon atoms of molecular weight 14. Chemically such a compound behaves like ordinary glucose and hence metabolically. When such a radioactive glucose is fed to an animal or used in a tissue slice experiment, the same chemical reactions take place as with ordinary glucose. The presence of ^{14}C in the tissue would indicate that the radioactive isotope must have been derived from glucose. The rates at which different substances are produced from glucose and the order of their synthesis can be followed by measuring the amount of radio-active carbon in different compounds. The Geiger-Muller radiation counter and scintillation counters constitute the instruments that are used for the determination of radio-active isotopes. Mass spectrometer is used for the determination of heavy and light isotopes which are not radioactive.

The radioactive isotopes used as tracers are forms of the element which do not occur naturally—they can therefore appear in a biological system only as a result of its introduction experimentally. 99.62 per cent of naturally occurring nitrogen in free or combined in chemical compounds is ^{14}N, ^{15}N being 0.38 per cent, the latter is often called heavy nitrogen. ^1H constitutes 99.98 per cent of hydrogen, ^2H accounts for 0.02 per cent, which is referred to as heavy hydrogen or deuterium with the symbol D. The radioactive isotopes can also be detected by means of the technique called autoradiography which is based on the ability of the emitted radiation to blacken photographic emulsion.

The rate of change f radioactive element to another element with emission of radiation indicates the rate of radioactive decay expressed in terms of half-life. A compound A containing radioactive isotope ^{14}C

when administered to an animal may produce the compound B which can be isolated, purified and its radioactivity determined. The compound B may undergo further breakdown and the ^{14}C activity associated with various carbon atoms may be determined. It often provides the information as to whether A constitutes a metabolic precursor of B and as to how the subsequent metabolic intermediates are formed. An example is provided by the administration of methyl-labeled ^{14}C acetate to rat when palmitic acid of high activity is isolated from the tissues, — COOH group or the β, δ carbon atoms having no activity but the α, γ carbon atoms with much of the activity. This indicates acetate to be the precursor of palmitic acid and the successive condensation of acetate results in the formation of palmitic acid with the addition of two carbon atoms to the chain — CO — groups being reduced to — CH$_2$-groups:

$$\overset{*}{C}H_3 - COOH + \overset{*}{C}H_3 - COOH \;\rightarrow\; \overset{*}{C}H_3CO - \overset{*}{C}H_2 - COOH \xrightarrow{\text{reduction}}$$
$$\;\;\alpha\qquad\qquad\beta\qquad\;\;\alpha\qquad\qquad\beta\qquad\qquad\;\;\alpha\qquad\quad\beta\qquad\quad\gamma$$

$$\overset{*}{C}H_3 - CH_2 - \overset{*}{C}H_2 - COOH + \overset{*}{C}H_3 - COOH \;\rightarrow$$
$$\;\;\alpha\qquad\;\beta\qquad\;\;\gamma\qquad\qquad\delta\qquad\;\;\alpha\qquad\quad\beta$$

$$\overset{*}{C}H_3 - CO - \overset{*}{C}H_2 - CH_2 - \overset{*}{C}H_2 - COOH \xrightarrow{\text{red}}$$
$$\;\;\alpha\qquad\;\beta\qquad\;\;\gamma\qquad\;\;\delta\qquad\quad\varepsilon$$

$$\overset{*}{C}H_3 - CH_2 - \overset{*}{C}H_2 - CH_3 - \overset{*}{C}H_2 - COOH \text{ and so on} \longrightarrow \text{Palmitic acid}$$
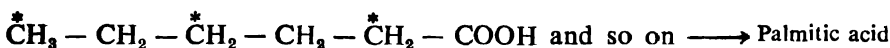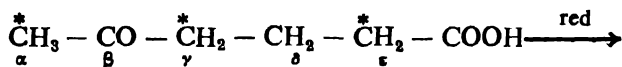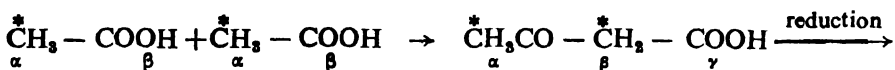
The rate of breakdown and synthesis or turnover of a substance in the body can also be determined by the application of isotopes as tracers. The rates of synthesis and breakdown of a substance balance each other so that its level in tissues may remain constant. The rates of breakdown and synthesis may be determined by the administration of isotopically labeled substance. Metabolic disturbances may be caused by disease, poisons, drugs or abnormal diets and are reflected in changes in the rates of synthesis and breakdown of a substance or substances in the body. Thus the increased rate of synthesis rather than the decreased rate of excretion of uric acid causes an increased turnover rate with elevated uric acid level in primary gout.

BLOOD AND TISSUE ANALYSIS

Catheterization permits withdrawal of blood samples from various levels of the vascular system and the determination of substances in the venous samples and those in arterial blood samples enables to follow the metabolic changes of substances due to the function of different organs. The catheterization technique has provided a means to study the utilization of oxygen, glucose and other substances by normal, and failing hearts, the synthesis of hormones by the adrenal glands, and other metabolic processes. Determination of tissue constituents, such as muscle glycogen in experimental animals, is necessary in metabolic studies.

ANALYSIS OF EXCRETIONS

Characteristic end-products appear in the urine and at times in the faeces as a result of metabolism of various substances in the body and useful information is provided by the analysis of the excretions. If an animal is fed on a diet containing 15 g of nitrogen as protein and the excretion amounts to 12 g the animal has retained 3 g of nitrogen in the form of 18.75 g (3 × 6.25) of tissue protein. The urinary sulphate increases promptly on ingestion of methionine indicating the breakdown of some of the methionine and the oxidation of sulphur to sulphuric acid in the body. Administration of lactic acid to diabetic patients results in excretion of much glucose in urine indicating the conversion of lactic acid to sugar in the body.

The nature and level of metabolic activity of substance can be determined by balance studies where the total intake of the substance in food and its total output or its metabolic products in all excretions are measured. The gross protein and mineral metabolisms have been elucidated with the help of balance studies.

RESPIRATORY EXCHANGE

Oxygen is consumed with the production of carbon dioxide during the oxidation of food stuffs in the body. The oxidation of carbohydrate (glucose), protein (amino acids), and fats (fatty acids and glycerol) gives rise to characteristic volume relations of these gases. The respiratory quotients, CO_2/O_2, are 1.00 for carbohydrate, 0.80 for protein, and 0.707 for fats. If the urinary nitrogen excretion indicating the amount of protein metabolized in given time and oxygen consumption and carbon dioxide production are measured, the oxidation of protein, carbohydrate and fat can be estimated quantitatively as well as the energy from each. The respiratory quotients of different organs and tissues may vary considerably at any given time and the additive effects of the metabolisms of the component tissues constitute the respiratory metabolism. A preponderance of fat oxidation is reflected in a respiratory quotient of 0.74, a RQ of 1.00 indicates that the brain is oxidizing glucose almost exclusively. Valuable information on metabolic studies are obtained by measuring the respiratory exchange of isolated tissues, tissue slices, and homogenates.

REMOVAL OF ENDOCRINE GLANDS AND OTHER ORGANS

Pancreatectomized animals and diabetic patients exhibit marked alterations of metabolic processes such as hyperglycemia (high blood sugar), glycosuria (sugar in urine), ketonemia (increased blood ketone bodies—acetone, acetoacetic acid, and β-hydroxy butyric acid), ketonuria (ketone bodies in urine), increased urinary nitrogen, low liver and muscle glycogen, and low respiratory quotients (RQ).

All these symptoms arise from insulin deficiency with profound changes in the metabolism of glucose, fatty acids, and amino acids. Blood sugar in completely diabetic animals continues to remain high even under conditions of fasting with urinary excretion of glucose and nitrogen at a relatively constant rate for considerable time. Ketone bodies continue to be exereted. Administration of alanine to these animals causes a prompt increase in urinary glucose indicating the metabolism of alanine through the pathway of glucose. Tyrosine on the other hand causes an increase in urinary ketones indicating the metabolism of tyrosine to proceed via acetoacetic acid formation.

For metabolic studies animals made diabetic by the administration of alloxan and similar substances, are used. The betacells of the pancreas responsible for insulin formation, are destroyed by alloxan. Administration of glucoside phlorizin destroys the glucose reabsorption capacity of the renal tubules; glucose thereby passes through the kidneys into the urine rapidly resulting in hypoglycemia and glycosuria. The tissues are able to use little sugar, the rate of protein and fat metabolism is greatly increased with ketosis. These symptoms are similar to those produced as a result of depancreatectomy. These diabetic animals are used to determine the metabolic pathway of a substance whether it is via glucose or acetoacetic acid.

Similar metabolic changes occur as a result of the removal of pituitary and adrenal glands. Injection of appropriate hormones into normal animals produces reverse of glandular removal and this procedure is extensively used in metabolic studies. Injection of adrenal cortical hormones into fasted normal rat causes increased blood sugar, liver glycogen and urinary nitrogen, indicating the involvement of the hormones in increased rate of formation of glucose and glycogen from the aminoacids of tissue proteins.

Metabolic studies particularly for gross overall effects, are also made by removal of organs such as kidney and liver in experimental animals.

Perfusion technique

Living organs after removal from the body, are used in metabolic studies by perfusing them *in situ* with blood or other fluids. Changes in the composition of the perfusing fluid containing various substances provide useful information in metabolic studies—liver perfused with alanine causes an increase in the perfusate indicating the involvement of liver cells in the deaminization of alanine to form pyruvic acid.

Warburg's tissue slice technique

Thin slices are made from fresh surviving tissues and placed in such media as Ringer's solution and the chemical changes produced on addition of certain substances are studied in the Barcroft-Warburg manometric apparatus. Respiratory exchage studies are possible by this technique. Amino acids are de-aminized by liver slices to form urea from the ammonia

produced. The metabolism of keto acids formed in the de-amination process can also be followed by this technique, which constitutes one of the most valuable methods for metabolic studies. Instead of tissue slices, finely macerated suspensions of tissues, called homogenates, are also used in vitro studies. Here the enzyme systems are liberated with cellular structure broken down.

STUDIES WITH PURIFIED ENZYME SYSTEMS

Purified enzymes provide a means for the studies of their catalytic reactions with the kinetic and energetic characterization. It also enables to determine the part played by the cofactors concerned, the inhibitory action of various substances, the influence of pH and temperature, and the presence or absence of essential reactive groups like the —SH in the enzyme.

The catalytic reaction of a purified enzyme in vitro provides important information but cannot indicate as to how the reaction occurs, is controlled and is related to other metabolic processes in the complex integrated living system.

Enzyme poisons

There are substances which are capable of inhibiting enzyme action selectively in a system containing several enzymes. Enzymes that are not inactivated in this way, can be studied better particularly when a number of enzymes are involved in the metabolism of a substance through successive stages. This becomes clear if a substance, A is considered, which is converted to D via the intermediate stages B and C. each stage being catalyzed by a specific enzyme. Poison or inhibitors can be chosen in such a way that the catalytic reaction at a particular stage can be inhibited without affecting the enzymes at other stages, resulting in the accumulation of the product at the stage where the enzyme action is inhibited. Iodoacetic acid poisons the muscle enzyme in such a way that glycogen breakdown cannot proceed beyond the stage of hexose phosphate.

Competitive inhibitor

Substances similar in structure to those required by organisms or analogous to the required metabolites, inhibit the enzyme action by combining with it for which the metabolite is a cofactor. This gives rise to physiological changes. p-amino benzoic acid is a cofactor for bacterial enzyme system.
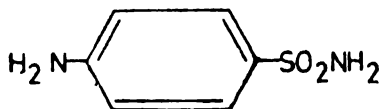
sulphanilamide   p – amino benzoic acid

Sulphanilamide has a structure similar to *p*-amino benzoic acid, whose cofactor part is taken over by the suphanilamide to exert its bacteriostatic action. The sulphanilamide thus acts as competitive inhibitor. Similarly pyrithiamine analogous to the vitamin thiamine, causes thiamine deficiency as it displaces thiamine in essential enzyme systems. All these techniques have provided important information in the study of intermediary metabolism in the living system.

Further Reading

E.A. Dawes, *Quantitative Problems in Biochemistry* (London: Livingstone, 1972).

E.H. Quimby and S. Feitelberg, *Radioactive Isotopes in Medicine and Biology* (London: Lea & Febiger, 1963).

D.Y. Hsia, *Inborn Errors of Metabolism* (Chicago: Year Book Publishers, 1959).

G.H. Bell, J.N. Davidson and D.E. Smith, *Textbook of Physiology and Biochemistry* (London: Livingstone, 1972).

E.S. West *et al.*, *Textbook of Biochemistry* (Delhi: Amerind, 1974).

EIGHT

Biological Oxidation: Bioenergetics

All biological systems—bacteria, plants and animals—require energy to carry out life functions. A portion of the energy constituting the visible segment of the electromagnetic spectrum or light, forms the energetic basis of all life on earth; the nuclear reactions occurring in the sun provide all the energy. The initial step in the conversion of energy by living organisms involves the absorption of light by the chlorophyll of green plants and some bacteria and the utilization of that energy to synthesize carbohydrates in the form of starch. This process is called photosynthesis. The products of photosynthesis in turn constitute the basic source of energy for all animal species, either directly or indirectly.

The manner in which the energy so derived is transduced or captured in a form unutilizable directly by biological systems and converted to a form capable of being utilized by biological systems to do work, is still not clearly known.

The chemical changes, like all other kinds of changes in the universe, obey and are governed by certain laws of thermodynamics—the branch of science which deals with energy and its transformations. The energy relations of system and its surroundings constitute the most fundamental rules of nature. The energy in biological systems needs the consideration of the general laws that govern those transformations and the application of thermodynamics to biological systems involves only the measurement of temperature and the chemical composition at the beginning and at the end of the reactions under consideration.

The laws of thermodynamics explain all chemical and physical events occurring in the universe under the control of the energy contained in the system and also as to how this energy is exchanged between the system and its surroundings. The part of the physical universe under study is referred to as system while the rest of the universe is called the surroundings. Energy is a fundamental physical concept and is roughly equivalent in meaning to work potential, or the capacity to do work. The energy

may be of different kinds—the potential and kinetic energies of mechanical systems, surface energy, pressure-volume energy, chemical energy, electrical energy, and radiant energy. The physical dimensions of energy are the same as those of work and can be represented by the formula: mass $\times$ (length)^2/(time)^2 or ML^2/T^2.

The first law of thermodynamics, also called the law of conservation of energy, states that, in any system, the sum of all energy changes must be zero, indicating that energy can neither be created nor destroyed. Thus the gain of energy in a chemical system must be balanced by the loss of that amount from the environment of the system. Conversely the loss of energy in a chemical system involves a gain of the same amount of energy by the environment. The first law therefore implies that energy can only be redistributed, changed in form, or both. The first law is concerned only with the initial and final states of the system and is independent of the details of the chemical or energetic pathway involved in the transformation from the initial to the final state. It is thus concerned with the difference between the initial and the final state.

The second law of thermodynamics states that any system, left to itself, tends towards a state of greatest stability. The actual stability of chemical systems depends on two factors mainly, both involving the energy of compounds. Enthalpy, designated by H, is one factor denoting the total energy content of a chemical system. A chemical compound is held together by chemical bonds that result from mutual electric attraction between atoms and ions. These bonding forces represent chemical energy, or bond energy. The bond energy increases with the increase in the attraction between two atoms or ions. When the external forces push them apart, the bond breaks with the bonded atoms or ions becoming disunited. Such forcible separation involves work of energy. Thus enthalpy or total chemical energy of a chemical compound may be defined as the energy required to break all the bonds in the compound. A chemical system with two or more compounds close together, will be most stable if and when its total chemical energy is at a minimum. Chemical reactions will therefore tend to proceed in such a way that, at the end, the total energy content of all the participants will be least.

'The simplest way of measuring the energy change involved in a chemical reaction is to determine the gain or loss of heat, called the enthalpy change denoted as ΔH. When the process occurs with the evolution of heat, it is termed exergonic or exothermic (if the energy is liberated in the form of heat). Decomposition reactions, including respiratory decompositions in living matter, belong to this category. When heat is absorbed, the process is endergonic or endothermic. For this type of reaction to occur, energy is required to be supplied from the environment to the reaction system. Synthesis reactions including respiratory decompositions in living matter, belong to this category. When heat is absorbed, the process is endergonic or endothermic. For this type of reaction to occur, energy is required to be supplied from the environment to the reaction

system. Synthesis reactions generally, including those in living matter, belong to the second category. This type of reactions tends to be expensive in terms of energy.

The second factor involved in the relative stability of a chemical system is its entropy, or energy distribution, denoted by S. A system is most stable when S is at a maximum with energy distributed as uniformly and randomly as possible. This state of randomness refers to what is called the entropy of the system—the greater the disorder, the greater is the entropy. Energy refers to work and heat; entropy refers to a form of energy which cannot do work and is equated with the degree of randomness of a system. The entropy differential is symbolized as ΔS. Entropy is temperature-dependent and the entropy changes are usually represented as the product of ΔS and $T . T$ being the absolute temperature at which a reaction occurs.

The overall stability change during a reaction is therefore dependent on two variables ΔH and $T \Delta S$. The stability changes as enthalpy increases and entropy decreases or as enthalpy decreases and entropy increases.

The first law of thermodynamics states that the energy lost by one side is gained by the other but it does not reveal anything about the direction in which the molecules or more generally, energy will flow. The second law of thermodynamics states that the energy flow will be in the direction of uniformity, or even distribution, or equilibrium.

All real processes are accompanied by an increase in entropy or randomness which would mean that the inevitable fate of the universe is to reach a state from which there is no deviation from randomness and disorder leading to what may be called entropic doom. Life however is just the opposite of randomness and it represents order.

The term energy in biological systems often refers to heat. If the heat is harnessed and used, the energy of heat is transformed into work:

$$\Delta E = q - w$$

ΔE denotes the change in the energy of the system, q is the heat increment, and w the amount of work done by the system. This is another way of expressing the first law of thermodynamics which states that the flow of heat is exactly counterbalanced by the energy change in the system or by work done by the system or by the combination of both. The energy change accompanying a chemical reaction is conveniently determined by measuring the gain or loss of heat, called the enthalpy change, ΔH. In biological systems there is no change in volume or pressure and if no work is accomplished, $\Delta H = \Delta E$. Each organic molecule has a characteristic heat of combustion and the amount of energy released as a result of the complete combustion of the molecule in presence of oxygen can be measured. An example is the complete oxidation of 1 mole of glucose in living cells:

$$C_6H_{12}O_6 + 6\,O_2 \;\rightarrow\; 6\,CO_2 + 6\,H_2O$$
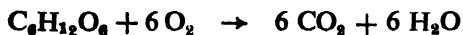
The molar enthalpy, or the heat evolved in the reaction is indicated by

$$\Delta H = -\,673{,}000 \text{ cal/mole}$$

The negative value for ΔH indicates that heat is lost from the system as a result of the reaction. The highly complex configuration of the organic molecule contains the energy from which the heat is derived. It is released in the formation of the simpler CO_2 and H_2O molecules. Oxidations out of the various types of chemical reactions taking place in living cells, release the greatest quantity of energy.

Heat is not converted into usable energy to any significant degree in biological systems which do not have significant temperature differences between one part of a cell and another, or one part of an organ and another. They are isothermal. Heat therefore cannot flow from one point to another. The energy flow in biological systems takes place in the form of another kind of energy called free energy. The concept of free energy is derived from the second law of thermodynamics which implies that all systems will tend toward equalization or equilibrium. The energy of a system which is capable of doing useful work under isothermal conditions is known as free energy also called Gibbs free energy, ΔG after Willard Gibbs. Free energy may be considered as useful energy and entropy as degraded or nonusable energy. The concept of free energy is particularly useful in biochemical reactions as it is a measure of the work that a reaction may do. The relationship among free energy, enthalpy, and entropy is indicated as

$$\Delta G = \Delta H - T\,\Delta S$$

ΔG denotes change in free energy, ΔH the change in enthalpy, which is equivalent to the change in the total energy of the system, T the temperature, and ΔS the change in entropy. This is an important equation representing a symbolic statement of the second law of thermodynamics.

Changes in free energy, enthalpy, and entropy are expressed as heat equivalents. The unit of heat is a calorie (cal)—the amount of heat or equivalent quantities of other energy forms, required to raise the temperature of 1 gram of water by 1°C. A commonly used biological unit is a kilocalorie (kcal) or dietary calorie, equal to 1000 cal.

A negative ΔG is characteristic of decomposition reaction and a positive ΔG, of synthesis. The synthetic reactions of the cell are essentially all endergonic, requiring an addition of energy to proceed. The synthesis of protein by the cell involves the building blocks, the amino acids, being put together. It is true that the amino acids are present as such in the cell but they are much more randomized in structure than the final product, the protein, representing a fantastic degree of order. The energy requirement for this endergonic reaction to proceed is obtained in the form of

free energy derived from a series of exergonic reactions, the two processes being coupled to one another. In living systems, actually decompositions are intimately coupled with syntheses, energy obtained from respiratory decomposition supporting metabolic synthesis.

The energetics of a chemical system thus determine one basic attribute of reactions, their direction. The second attribute. rate, is specified by factors that influence the collision frequency among chemical units. The endergonic reaction will not be possible unless the decline in the free energy of the exergonic reactions is greater than the increase in free energy of the endergonic reaction. The algebraic sum of the free energies of the net reaction must be negative.

Activation energy is still another important form of energy in biology. It is a property of enzymes, the biological catalysts that increase the rate of a chemical reaction without altering the equilibrium of that reaction, The chemical reactions occurring in the cell, proceed thousand of times more rapidly in presence of enzymes than in its absence, even when the reaction is quite exergonic. The reason is that an energetic barrier, the activation energy, must be overcome.

ACTIVATION ENERGY: EFFECT OF ENZYME FOR A REACTION

From Fig. 8 i and Fig. 8.2 we find that only a small part of the total number of molecules of compound A contains enough energy to react at any particular time. The energy level of the molecules would be increased with the application of heat in direct proportion to the heat applied and a larger proportion of the molecules in consequence would overcome the activation energy barrier. The molecules of the compound A also get activated by the enzyme by forming an unstable enzyme-substrate complex raising the energy level of each substrate molecule A and renders it more susceptible to reaction. The enzyme does not alter the change in free energy of the reaction indicated arbitrarily by − 1000 cal in the Fig. 8.1 and
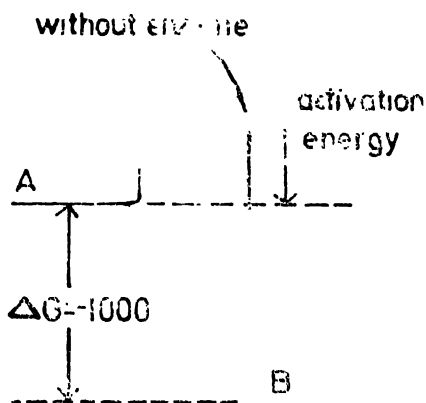
Fig. 8.1

Fig. 8.2. The equilibrium of the reaction is not altered by the enzyme which only makes the reaction to reach equilibrium much more rapidly.
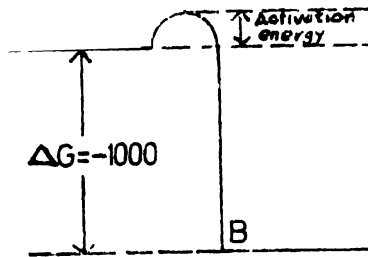
Fig. 8.2
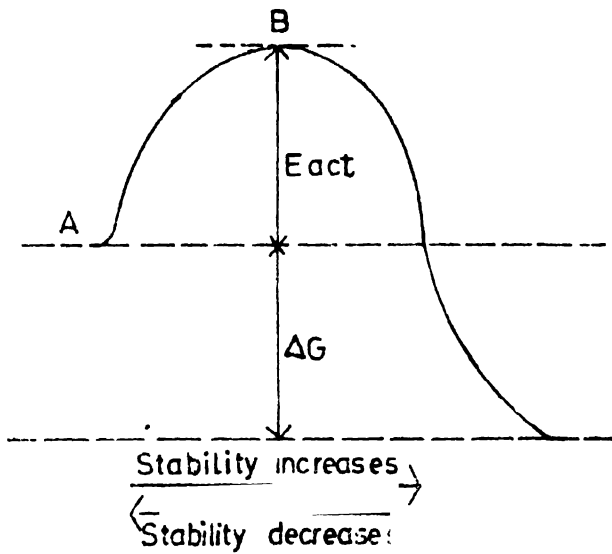
Fig. 8.3

In Fig. 8.3, an exergonic reaction from A to C, when A is less stable chemical system than C, can take place in case the activation energy, E_{act}, is applied first to A. E_{act} brings the reacting system over an energy barrier B, with the release of energy as A changes into C. E_{act} expended earlier is paid back as a result of this energy release, yielding additional energy, ΔG, the free-energy change. The reaction leads to the net energy gain, ΔG which varies in proportion with the stability difference between A and C. In an endergonic reaction from C to A on the contrary, C has not only to be activated but also to be provided with external energy amounting to ΔG. In this case E_{act} represents the only energy gain the net energy expenditure being ΔG.

Most chemical reactions proceeding in biological system need fairly high activation energies. Fat and water mixture remain practically un-changed for days at room or body temperature. In the body they react

appreciably within an hour or so, as catalysis produces high rates of molecular collisions within the living body without the application of additional heat, reactions are accelerated by catalysts (enzymes) instead of heat. The catalysts occurring in living matter are called enzymes, which are all proteins, each being characterized by its specificity. The effect of enzymes is to lower the requirements of activation-energy, promoting appreciable reaction rates at lower temperatures than would be possible otherwise. Reactions become faster due to mutual contact between the enzyme and the substrate (the substance on which the enzyme acts) forming what is called the enzyme-substrate complex as a lock-and-key process.

The whole surface of the enzyme is often not required to promote a reaction because the enzyme proteins are huge molecules compared to most substrates. The active sites of the enzyme protein are only involved. Enzyme specificity results from the differences in the surface configuration of different protein types—a particular type of enzyme accelerating only one particular type of reaction.

The concept of active sites is consistent with the idea that enzyme molecules are flexible and that the structure of a particular substrate can induce the enzyme to bend or mould itself over the substrate. This also explains the observation that many enzymes need cofactors, such as metal ions, Mg^{++}, Ca^{+++}, etc., which aid in moulding an enzyme or its substrates into the shape required for a proper enzyme-substrate fit.

Changes of temperature, pH, and environmental conditions influence the effectiveness of enzymes, because of their protein nature. The optimal temperature range for most enzymes lies between 25 to 40°C (37°C is human body temperature) and a pH range between 6.0 to 7.5.

THE ENERGY CURRENCY OF THE CELL—ADENOSINE TRIPHOSPHATE (ATP)

The exergonic and endergonic reactions and the coupling of energy between them are absolutely essential for the existence of biological systems. The exergonic reactions provide the cell with its usable energy. The heat released in many of the exergonic reactions of the cell is trapped in a form of chemical energy capable of doing useful work and driving endergonic reactions like the synthesis of protein, carbohydrate, fat, etc., in the cell. Adenosine triphosphate, ATP, contains the energy currency of the cell. The ATP consists of the nucleoside adenine, the five-carbon-sugar D-ribose and the three phosphates attached to D-ribose through α-phosphate. The α- and β- and the β- and γ-phosphates are connected through ester or acid anhydride linkages, called the high energy phosphate bonds ($\sim P$), indicating that considerable free energy becomes available to do useful work as a result of energy release during the splitting of the phosphate ester linkage of ATP. Approximately −7000 cal/mole become available as a result of the coupled splitting of the terminal phosphate of ATP under physiological conditions.

The major components of food, carbohydrate, fat, and protein, in the course of their respective metabolic breakdown undergo exergonic reactions to release usable energy that may be trapped in the form of ATP. One of the principal functions of glycolysis is to prepare carbohydrates to enter the citric acid cycle and the electron transfer chain for the complete oxidation of glucose to carbon dioxide and water $(C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O)$ through a system of enzymes and cofactors. The major metabolic interrelationships in the cell are indicated in Fig. 8.4.
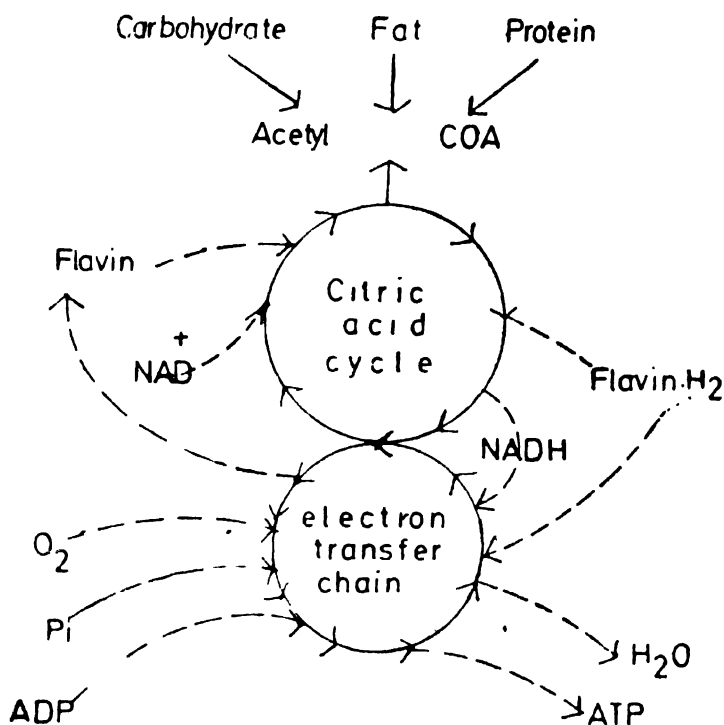
Fig. 8.4

Transformation of 1 mole of glucose to 6 moles of CO_2 and 6 moles of H_2O under aerobic conditions, involves the standard free-energy change of $-686,000$ cal. Aerobic glycolysis provides, a free-energy change of $-50,000$ cal, about 7 per cent of the total. Subsequent processes associated with the citric acid cycle and the electron transfer chain, account for the remainder of the free-energy change about 93 per cent. The major function of glycolysis, under aerobic conditions, is thus to prepare the major energy substrate, glucose, for entering the metabolic sequences which release the major fraction of the energy contained in the glucose molecule. The free-energy change associated with glucose oxidation is shown overleaf;

Glucose

Glycolysis |
↓   ~50,000 Cal

2 Pyruvate

Citric acid
cycle and
electron
transfer chain

υ.O₂

6 CO_2 + 6 H_2O

**Standard
Free energy
change**

$-686,000$ Cal.
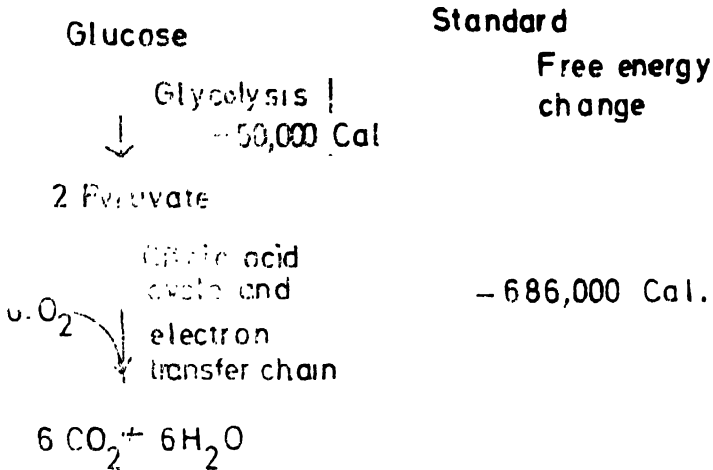
Simple six-carbon sugars are formed from complex carbohydrates like glycogen and starch in the intestine and transported to the cell by the blood as glucose which is required to be converted to glucose-6-phosphate first by the enzyme hexokinase:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ATP}$$
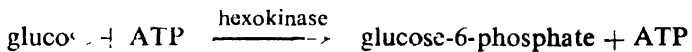
The reaction is endergonic requiring one molecule of ATP, the γ-phosphate being transferred to the number six carbon of glucose.

Adenine

Adenosine

D-ribose

Adenosine triposphate (ATP)
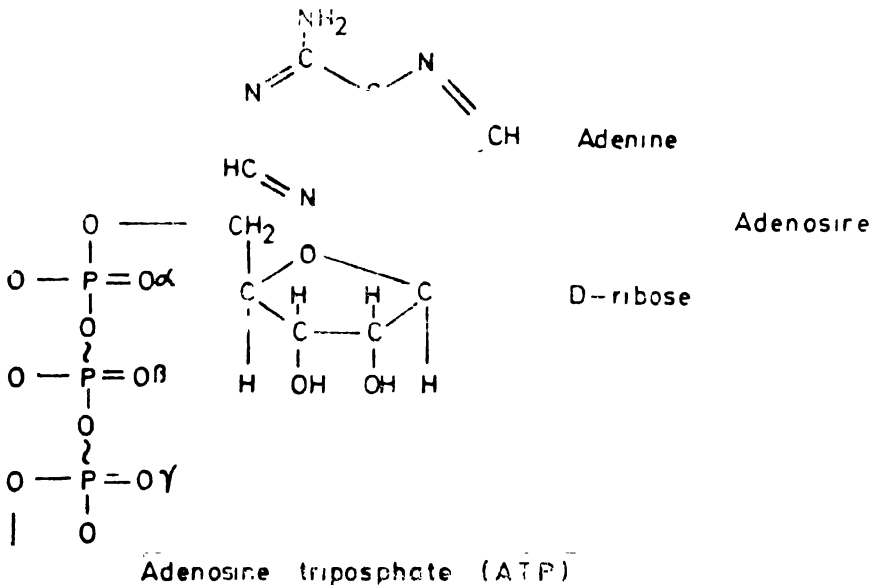
Glucose-6-phosphate is transformed into fructose-6-phosphate in the

next sequence of reaction which is exergonic and does not require energy input:

$$\text{glucose-6-phosphate} \xrightarrow{\text{phosphoglycomutase}} \text{fructose-6-phosphate.}$$
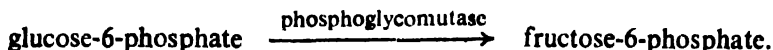
Fructose-6-phosphate undergoes phosphorylation again by another molecule of ATP, enzyme involved being phosphofructokinase:

$$\text{fructose-6-phosphate} + \text{ATP} \xrightarrow{\text{phosphofructokinase}} \text{fructose-1, 6-diphosphate} + \text{ADP.}$$
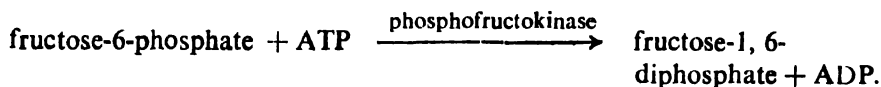
The phospho-fructokinase reaction is the most important step in glycolysis in regulating the carbohydrate metabolism. The reaction is irreversible proceeding in one direction only as shown. The cell under certain circumstances, converts some amino acids to glucose using in the reverse direction all the enzymes of glycolysis except phosphofructokinase. A different enzyme, fructose-1, 6-diphosphatase, is involved in the catalysis of the reverse reaction:

$$\text{fructose-1, 6-diphosphate} \xrightarrow{\text{fructose-1, 6-diphosphatase}} \text{fructose-6-phosphate} + P_l.$$
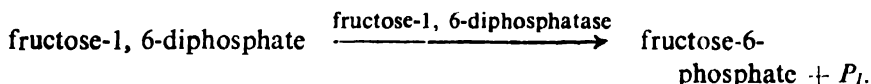
The cell thus regulates the breakdown and the synthesis of glucose independently. The phosphofructokinase reaction is inhibited when the breakdown of glucose has produced a high level of ATP in the cell. On the other hand fructose-1, 6-diphosphatase reaction is inhibited when the cellular concentration of ATP is low and when AMP, one of the products of ATP utilization, rises in concentration. Thus the energetic requirements of the cell determine the rate of both breakdown and synthesis of glucose. Next the six-carbon-diphosphorylated sugar is broken down into the two three-carbon phosphorylated molecules, 3-phosphoglyceraldehyde and dihydroxyacetone phosphate:

$$\text{fructose-1, 6-diphosphate} \xrightarrow{\text{aldolase}} \text{3-PGA} + \text{DHAP}$$
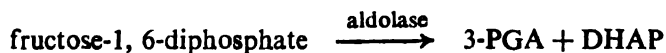
Aldolase reaction gives rise to dihydroxyacetone phosphate (DHAP) as one of the two products. The enzyme triose-phosphate isomerase is able to convert DHAP to 3-phosphoglyceraldehyde (3-PGA). Thus the remainder of the glycolytic pathway enzymes catalyse both the triose phosphate compounds identically. The enzyme glyceraldehyde phosphate dehydrogenase in presence of inorganic phosphate (P_i) and the oxidized form of the coenzyme nicotinamide adenine dinucleotide (NAD) acts on both 3-phosphoglyceraldehyde molecules to form two molecules of 1, 3-diphosphoglycerate and two molecules of the reduced form of the coenzyme, NADH:

$$2 \, (3\text{-PGA}) + 2P_i + 2NAD \xrightarrow{\text{glyceraldehyde-P-dehydrogenase}} 2 \, (1, 3\text{-diphosphoglycerate}) + 2NADH$$
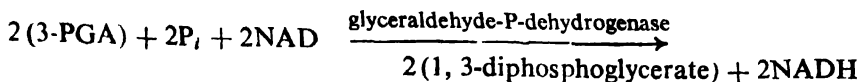
This reaction is of importance to the cell; it adds another phosphate to the molecule and prepares it for the synthesis of a molecule of ATP. The removal of hydrogen from 3-phosphoglyceraldehyde results in some energy release which is conserved in the form of reduced coenzyme, NADH, instead of being released as heat. This coenzyme plays a key role in the energy transduction process.

Two molecules of 1, 3-diphosphoglycerate and two molecules of ADP are transformed in the next reaction sequence to two molecules of 3-phosphoglycerate and the synthesis of two molecules of ATP by the enzyme diphosphoglycerate kinase:

$$2 \, (1, 3\text{-diphosphoglycerate}) + 2ADP \xrightarrow{\text{diphosphoglycerate kinase}} 2 \, (3\text{-phosphoglycerate}) + 2ATP$$
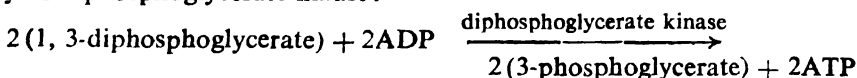
Here two inorganic phosphates activated by the previous reaction, are transferred to ADP to form two important molecules with high-energy bond or high-group transfer potential, ATP. The two molecules of ATP used previously in the glycolytic process at the hexokinase and phosphofructokinase steps are released and the cell has now become even in an energetic sense.

The position of the phosphate on the glycerate is rearranged in the next two reactions with the elimination of a molecule of water from the glycerate to form a molecule of phosphoenol-pyruvate containing a high energy phosphate because of its new double-bonded resonance form:

$$2 \, (3\text{-phosphoglycerate}) \xrightarrow{\text{phosphoglucomutase}} 2 \, (\text{phosphoglycerate})$$

$$2 \, (2\text{-phosphoglycerate} \xrightarrow{\text{enolase}} 2 \, (\text{phosphoenolpyruvate}) + 2H_2O$$
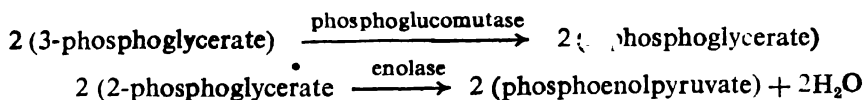
Phosphoenolpyruvate then reacts with ADP to form ATP and pyruvate:

$$2 \, (\text{phosphoenol pyruvate}) + 2ADP \xrightarrow{\text{pyruvatekinase}} 2 \, (\text{pyruvate}) + 2ATP.$$

Thus ends the chain of reactions, known as aerobic glycolysis, or the Embden-Meyerhof pathway.

This pathway to the cell is of importance in many respects. Less than 10 per cent of the free energy available from glucose is released and the molecule is changed to pyruvate for entering into the major energy-releasing machinery of the cell, the citric acid cycle and the electron transfer chain. In the process two molecules of ATP are utilized with the synthesis of four molecules of ATP—a net gain in the synthesis of two ATP molecules. Simultaneously the glyceraldehyde phosphate dehydrogenase reaction generates two molecules of NADH, the metabolism of

which is also a strongly exergonic reaction. One mole of glucose is capable of free-energy change of $-686,000$ cal out of which about 50,000 cal are released in the process of glycolysis. The molecular alterations taking place in the course of citric acid cycle and oxidation by the electron transfer chain account for the remainder of the free-energy change which are released subsequently for the series of complex reactions collectively known as the citric acid cycle and oxidative phosphorylation.

The enzymes bringing about the glycolysis, exist in the soluble portion of the cytoplasm. The enzymes of the citric acid cycle, the electron transfer chain and fatty acid oxidation alongwith the structural elements, such as protein and lipids exist in a highly ordered geometric sequence within an organelle of the cytoplasm of the cell, the mitochondrion. The citric acid cycle is also known as the tricarboxylic acid cycle and the Krebs cycle after Sir Hans Krebs who received the Nobel Prize for this work on the citric acid cycle enzymes. The cycle may be illustrated as in Fig. 8.5.

Pyruvic acid, the product of glycolysis, can cross the outer mitochondrial membrane and enter the mitochondrion for the enzyme pyruvic dehydrogenase to act on it in a number of ways. The enzyme first splits a carbon from pyruvic acid leading to the production of carbon dioxide and the two carbon acetic acid. It is concerned with the conservation of most of the energy released by the carbon-to-carbon bond split by linking the acetic acid to coenzyme A through a sulphur atom of the coenzyme A resulting in the formation of Acetyl $\sim$ CoA. In addition the enzyme catalyses the transfer of hydrogen from pyruvic acid to the primary hydrogen acceptor, nicotinamide adenine dinucleotide (NAD) to form NADH (reduced form of NAD). NAD is structurally represented as in Fig. 8.6.

The high-energy acetyl $\sim$ CoA enters the citric acid cycle by combining with a four-carbon molecule, oxaloacetic acid to form citric acid, CoA is released and once again takes part in the introduction of more two-carbon units into the citric acid cycle. Isocitric acid is formed through the intermediate cis-aconitic acid. An important energetic step in the cycle is the conversion of isocitric acid to α-Ketoglutaric acid. At this step one carbon is lost and another molecule of NADH is formed. Still another NADH is formed in the conversion of α-Ketoglutaric acid to succinyl CoA. This step is also important in energy-conservation, succinyl CoA being energy-rich.

At the next step, conversion of succinyl CoA to succinic acid results in the formation of ATP. The energy-conservation at this stage happens as a result of the breaking of the high-energy succinyl CoA and the utilization of the free-energy change to bond inorganic phosphate (P_i) to the enzyme catalyzing the reaction. The high-energy enzyme-phosphate intermediate transfers the phosphate to ADP to form ATP. Succinic acid, the four-carbon citric acid cycle intermediate, resulting from this reaction participates in an energy-conservation step in a different manner by its conversion to fumaric acid when hydrogen is transferred from succinic

Glucose

↓

Glucose-6-phosphate

↓

Glycolysis

↓

Pyruvate

↓

Citric acid cycle

Pentose phosphate pathway

↓

$CO_2 + H_2O$

→ CO_2

2H

↓

Terminal electron transport Patwhay

→ H_2O

NH₂   Adenine
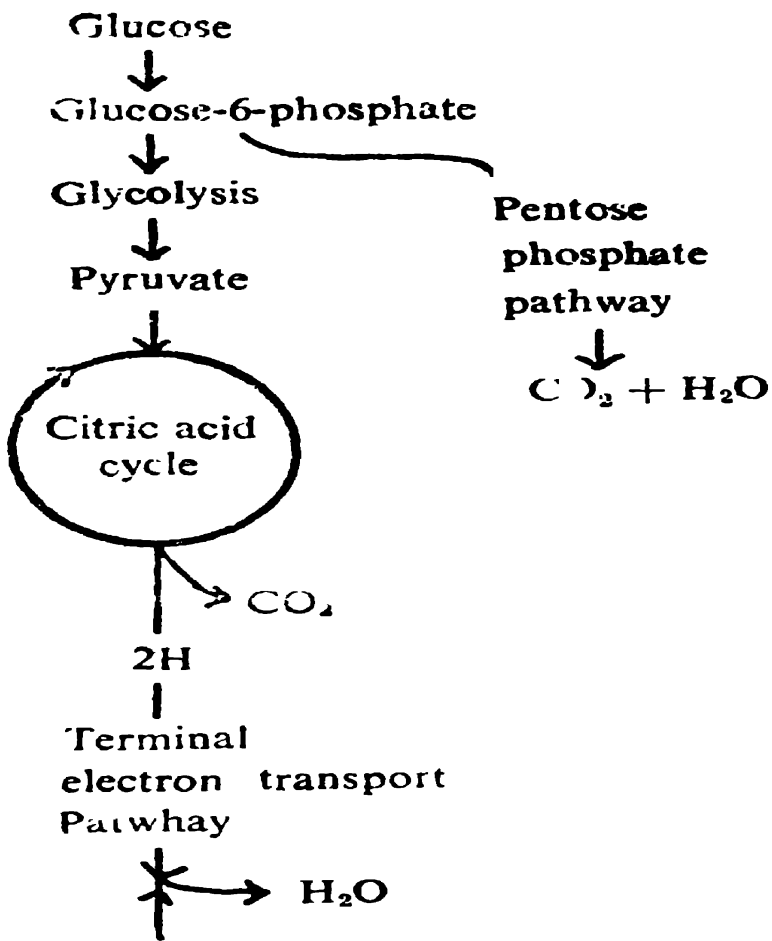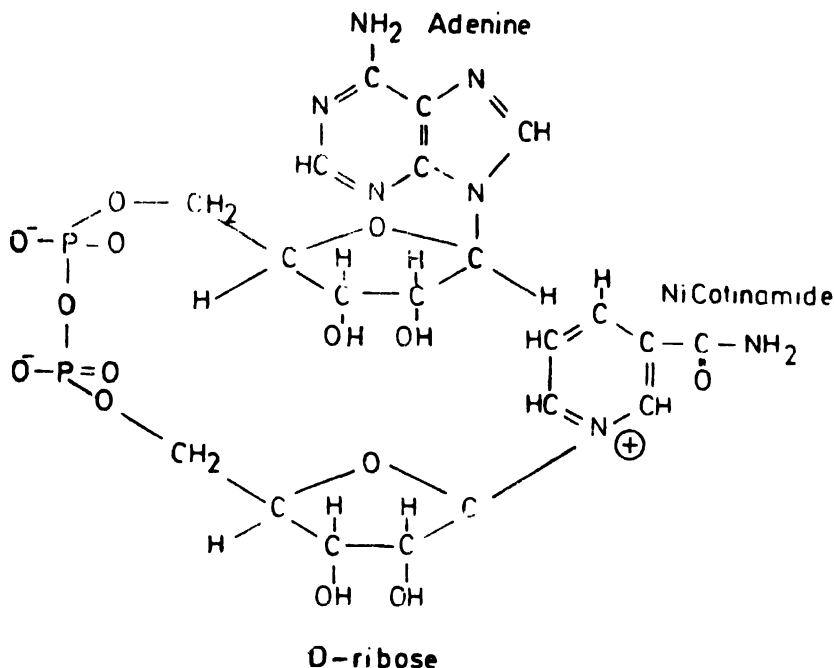
Fig. 8.6   Structure of NAD.

acid by its enzyme to its enzyme.   The enzyme involved, succinic dehydro-
genase, contains a group, called flavin adenine dinucleotide (FAD),
capable of accepting hydrogen and thus being raised to a higher free-energy
level.   Oxaloacetic acid is formed again in the last reaction of the cycle to
come back to the starting step.

Succinic acid and NADH, two of the products ˙ the citric acid cycle,
function as substrates, or energy donors, for the electron transfer chain.
They are hydrogen rich being in the reduced forms, and can therefore
donate hydrogen, or electrons to hydrogen-poor (oxidized) molecules with
electron-accepting groups.   The two enzymes, flavoprotein dehydrogenases
named as succinic and NADH dehydrogenases, catalyse the transfer of
electrons from the high-electron-potential molecules to the chain regenera-
ting the oxidized forms of the substrates, fumarate and NAD, so that the
citric acid cycle and glycolysis can proceed and donate free energy to the
electron transfer chain.

Because of the lower electron potential of the successive components
of the electron transfer chain, each is capable of accepting electrons from
the immediately preceding component until the component with the lowest
electron transfer potential, atomic oxygen ($\frac{1}{2}O_2$), accepts a pair of electrons
and combines with a pair of hydrogen ions to form water.   Energy is
released at each step with the transfer of electrons along the electron
transfer chain from component to component.   Three molecules of ATP
are synthesized by the transfer of a pair of electrons from NADH to

oxygen. Only two molecules of ATP are formed by the oxidation of succinic acid. The transfer of electrons from molecules of higher to lower electron potential constitutes a series of exergonic reactions which drive a series of endergonic reactions, the synthesis of ATP from ADP and inorganic phosphate. This process is called oxidative phosphorylation.

The principal function of the oxidative phosphorylation appears to be the conservation of energy and the amount of free energy released due to transfer of a pair of electrons from 1 mole of succinic acid or NADH to oxygen can be calculated using the electron potential (or oxygen-reduction potential) difference between major components of the electron transfer chain.

Many biological processes are the results of coupled reactions, the first of which supplies the energy that allows the spontaneous occurrence of the second reaction. ATP is the energy supplier in these processes and gets degraded to ADP and phosphate. The 'high energy' of ATP may be transferred to a substrate involving either phosphate or adenyl group. It is for this the ATP is considered to be the energy currency in many biochemical reactions.

$$\begin{array}{llll} \text{ATP} & \rightarrow & \text{AMP} + \text{P}\sim\text{P} & \Delta G < 0 \qquad (1) \\ \underline{\quad X \quad} & \rightarrow & \underline{Y} & \underline{\Delta G > 0} \qquad (2) \\ \text{ATP} + X & \rightarrow & X\sim\text{AMP} + \text{P}\sim\text{P} & \\ X\sim\text{AMP} & \rightarrow & Y + \text{AMP} & \Delta G < 0 \qquad (3) \end{array}$$
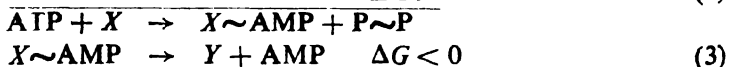
Reaction (3) represents the coupled reaction of (1) and (2) involving group transfer from (1) to (2). The total ΔG for $X \rightarrow Y$ is favourable due to activation of X by AMP.

The free-energy change of a chemical reaction decides as to whether the reaction is thermodynamically favourable or not. At equilibrium,

$$\Delta G = 0 \quad \text{and} \quad \Delta G^\circ = -RT \ln K$$
$$\Delta G^\circ = -RT \ln K \quad \text{cal per mole.}$$

Where R, the gas constant $= 1.987$ cal per mole per degree; T, the absolute temperature; and $\ln K =$ the natural logarithm of the equilibrium constant $(2.303 \log K)$. Thus the determination of the equilibrium constant of a reversible reaction enables the calculation of the standard free energy of the reaction (ΔG°). Conversely ΔG° provides the means to determine the equilibrium constant.

Generally the reactants and products of a biochemical reaction do not fall under standard state activities and the free-energy change, ΔG, of the reaction taking place at the concentrations (activities) present in a tissue can be calculated for a reaction:

$$A + B \ \rightleftarrows \ C + D$$
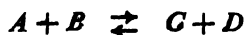
The free-energy change (ΔG) and the standard free-energy change (ΔG°) are related :

Preface

Biochemistry came to be known at the advent of the present century and has now developed into a vastly complex science with unabated pace. The remaining part of this century may be the 'Age of Biology'. Biochemical genetics and molecular biology formed a part of the curriculum a decade or so ago. The emergence of new subjects of current relevance—cell biology, development, inheritance, animal behaviour, ecology and evolution—present a selection of important topics today.

New knowledge evolves from old and a blending of the two enables the student to follow the course of this spectacular development. Man as a living organism has so much in common with all other life that he has to live in harmony with the environment. Students of today will face some of the gravest problems of tomorrow, biological in nature—population explosion, adequate or inadequate natural resources, pollution, environmental health and the host of their natural successors—bringing in their wake economic and sociological problems.

The Krebs-cycle brought about a revolution in the understanding of the molecular outlook and must be retained as a necessary foundation for a presentation of modern biology with its present chemical and biological shift at the cellular level. Biochemistry is based considerably on the principles of physical and organic chemistry. The basic sequence of topics in the book has been followed with that objective in view broadly pertaining to organization and operation of living matter. It provides a scientific, biological, and chemical background. A wholesome approach to contemporary biochemistry cannot escape a balanced emphasis on molecules, cells, organisms, population, communities, and ecosystems. It has also to stress the interplay of the great unifying principles of biology at all these levels of the organization of life. An historical oreintation enables the student to understand as to how the present state of knowledge has been attained. It is an essential part of the wisdom needed to continue into the future.

Repetition of important facts and theories has been a common feature throughout the text with the belief that such repetition is invaluable to the learning process. The so-called facts which the student learns are at least five vears out of date when he meets them and will be almost

viii PREFACE

obsolete after ten years—the progress of science being so terrific with a doubling period of about ten years. It has been said that the value of any fundamental education lies in the attitude which remains when the facts have been forgotten.

A textbook has to be interesting, accurate, educationally valuable and, in particular, has to be adapted to the state of knowledge and development of readers. Students are more often handicapped by lack of understanding rather than by lack of information.

Medical students studying biochemistry require two types of facts—those that lead to principles and those that, while not yet leading to a principle, are nevertheless important for clinical medicine. Biochemistry can help a medical student to develop an attitude which will be helpful throughout life and which will not be dependent upon the memory of the facts learned during the preclinical years by showing him how to deal with biological problems, how to pick his way amongst conflicting information, and how to use that information to build up a coherent picture. Not often it occurs to medical students that their knowledge of biochemistry can be used in a constructive way after all the examinations are over.

I have tried to present an account in a way which users are likely to find clear, helpful and interesting, an account which seeks to develop understanding and a critical approach. There are few areas where current research is both exciting and intelligible which I have ventured to discuss even though it may not be relevant to the students' examinations. There is no better way to encourage a student to think of the working of the body as a whole than to ask him to study the response to various diverse environments. It is all too easy to think of a perfused, isolated kidney or a liver slice in the laboratory without making an attempt to relate it to the life of the intact animal.

I have felt in the course of my association of over two decades with medical education the absence of a text which could encourage a systematic study of the life processes and yet retain the interest and understanding in a coherent manner. At the close of my career the idea of the book was almost thrust on me by a close friend of mine.

I do not know how to express my gratitude to my friend who inspired me to undertake this venture. He is no more with us today.

At every step I had the good fortune to receive guidance and material help in abundance from a large number of my distinguished colleagues and friends; but for their help I could not have been in a position to undertake this task. My grateful thanks are due to all of them.

S. K. DASGUPTA

Contents

$$\Delta G = \Delta G^\circ + RT \ln \frac{[C].[D]}{[A].[B]} \text{ cal per mole.}$$

The standard free-energy change (ΔG°) can be calculated from the equilibrium constant of a reaction and the free-energy changes (ΔG) for any concentrations (activities) of reactants and products are determined from the foregoing equation.

At equilibrium no further change in concentrations of reactants and products takes place with no change in free-energy in the reaction ($\Delta G = 0$). No work can be obtained under such equilibrium. The maximum free-energy change in a reaction occurs when the activities of reactants and products are farthest from the equilibrium state and a decrease in free-energy ($-\Delta G$) ensures that the maximum work is obtainable under these conditions.

Reactions with free-energy decrease ($-\Delta G$) of the system are called exergonic indicating the flow of free-energy from the system (capacity to do work decreases). Reactions with free-energy increase ($+\Delta G$) of the system are endergor'c.

Living organisms tend to maintain biochemical reactions away from equilibrium so that the reactions may provide the free-energy ($-\Delta G$) to support the living processes. This is done by the intake and oxidation of food and the excretion of waste products. Biological oxidation is both aerobic (in presence of air) and anerobic (no air). Anerobic oxidation is, in fact, dehydrogenation. The organism is dead when the reactions within it are at equilibrium. A free-energy change ($-\Delta G$) of about -7000 cal is necessary to bond 1 mole of ADP to 1 mole of inorganic phosphate (P_i) to produce 1 mole of ATP. The net ATP production during glucose oxidation is indicated in Table 8.1.

TABLE 8.1

Sequence	Net ATP molecules produced
Glucose → 2 pyruvic acids	2
2 pyruvic acids → $6CO_2 + 6H_2O$	36

Conversion of one molecule of glucose to two molecules of pyruvic acids results in a net production of two molecules of ATP. The conversion of two molecules of pyruvic acid to six molecules of carbon dioxide and six molecules of water leads to a net production of 36 molecules of ATP. Glucose oxidation thus gives rise to 38 molecules of ATP, the glycolytic pathway contributing only 5 per cent of the total conservation of energy. The aerobic process of oxidative phosphorylation thus constitute the major energy-transducing mechanism of the cell. The overall efficiency of the conserving process during the cellular oxidation of glucose is indicated by the fact that the free-energy change of glucose oxidation is $-686,000$

cal/mole and the free-energy change involved in the synthesis of 1 mole of ATP is 7000 cal. The efficiency in per cent is:

$$\frac{7000 \times 38}{686,000} \times 100 = 39 \text{ per cent.}$$

This is the minimum efficiency since the free-energy value of -7000 cal per mole for ATP synthesis may rise to 12,000 cal per mole, depending on the actual concentrations of ADP, ATP and phosphate at the actual site on the enzymes catalyzing the reaction whereby the efficiency could be about 60 per cent. These values for energy transduction indicate, in energetic terms, the cell's work efficiency.

The oxidative phosphorylation is the major energy conserver in the cell. The mechanism of the coupling between the exergonic process, the electron transfer, the endergonic process and the ATP synthesis, remains as yet unknown. Electron-transfer-supported ATP synthesis was discovered in 1939 and the chemical coupling hypothesis was postulated 15 years later. It is based on chemical considerations only suggesting that a 'high-energy intermediate' is formed from the free-energy released during electron transfer reactions as indicated:

$$\text{electron transfer} \to \sim \; \to \; \sim P \to ATP$$

Here the first-energy intermediate is not phosphorylated and the next high-energy intermediate is phosphorylated. This is supported by some indirect evidence. With the use of isolated pieces of the inner mitochondrial membrane, called submitochondrial particles, which can carry out oxidative phosphorylation, it has been found that energy from ATP may be utilized to push electrons up from succinate to NAD. This is the reverse of oxidative phosphorylation. Energy from ATP is used to reduce rather than to oxidize a substrate with high-electron transfer potential.

PHOTOSYNTHESIS

The glucose metabolism occurs in cells in the mitochondria. A cell can carry out metabolic synthetic activities once nutrients and ATP are available. Photosynthesis is the process whereby green plants utilize the energy of light for the synthesis of organic compounds through the transformation of inorganic CO_2 and H_2O, specially carbohydrates. These compounds are the primary substances from which almost the entire organic part of the living world is constructed, and on which virtually all organisms depend for their food. The chemosynthetic bacteria constituting probably less than 0.0001 per cent of all the living matter on earth, are the only organisms which are not dependent on photosynthesis. The solar energy harvested annually through photosynthesis in the form of carbohydrates amounts to one-fourth of the total energy now available to man from all sources.

Electrons and hydrogen ions (H^+) are required to be added to the carbon atom for the conversion of CO_2 to carbohydrate. CO_2 does not react directly with water. In photosynthesis CO_2 reacts with the hydrogen of water leaving its oxygen as a byproduct. Decomposition of water molecule into hydrogen and oxygen components constitutes the first step in photosynthesis. This decomposition is associated with processes involving the green pigment chlorophyll and the energy of light. This first phase of photosynthesis is called photolysis, or light-associated water decomposition. The hydrogen resulting from photolysis reacts with CO_2 in the second phase, to form a carbohydrate. This phase is called CO_2 fixation, implying a combination of CO_2 and hydrogen. Unlike photolysis, CO_{2-} fixation does not require light.

Thus the two phases of photosynthesis are photolysis and CO_2 fixation, both taking place in the grana of chloroplasts.

Photolysis
(light reaction)

CO₂ fixation
(dark reaction)

$$light \xrightarrow{\text{energy}} chlorophyl$$

$$2H_2O \xrightarrow{\text{energy}} 2H_2 \quad O_2$$

$$CO_2 + 2H_2 \longrightarrow [CH_2O] + H_2O$$

Carbohydrate
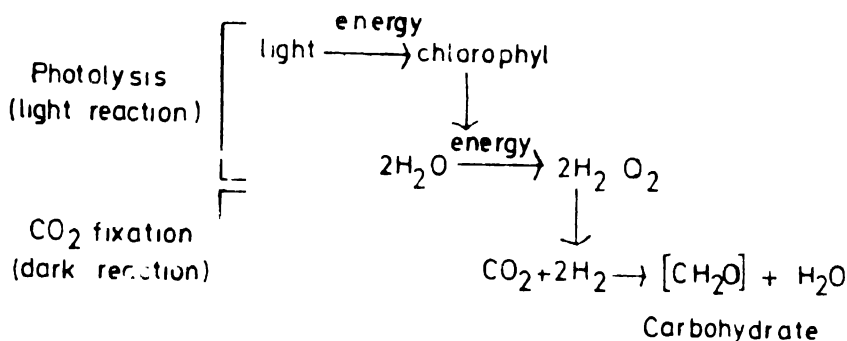
The chemical reduction of CO_2 requires energy. Radiant energy absorbed by the chloroplast pigments indirectly provides this energy. The complete photosynthetic machinery of higher plants is contained within a membrane-bounded organelle called a chloroplast. A single green cell contains from 1 to about 80 chloroplasts and the total surface area for light absorption of all the chloroplasts in a mature tree can be in the range of about 150 square miles. A chloroplast contains numerous grana, the usual structural units for photosynthesis.

Each granum of a chloroplast is a stack of protein layers and the chemical machinery of photosynthesis is located in the spaces between these layers. This machinery consists of enzymes, carrier coenzymes for hydrogen and other substances, nucleic acids (DNA and RNA), lipids, and three kinds of pigments, carotenoids, xanthophylls and chlorophylls. The grana disks of thylakoids are distributed in the region called the stroma which is an aqueous or soluble phase containing many proteins (enzymes) involved in the metabolic transformations of sugars and starches as well as other biochemical activities, such as protein synthesis and nucleic acid (DNA and RNA) metabolism. The thylakoid membranes contain the light-absorbing pigments—chlorophyll and carotenoids, and proteins

required to facilitate the oxidation of water and the transfer of electrons to an electron acceptor, which is used to donate the electrons to CO_2 reducing the carbon compound to carbohydrate. The thylakoid membrane consists of 50 per cent lipid (fatty compounds) and 50 per cent protein.

A molecule of chlorophyll consists of a head and a tail, the head containing four carbon-nitrogen rings joined together with a single atom of magnesium at the centre. The tail is a chain of linked carbons attached to the head. Different structural forms of chlorophyll exist in various phyla of photo-synthetic organisms. Chlorophyll$_a$ occurs almost universally in photo-synthetic organisms, suggesting that this particular pigment is essential for photolysis.

The molecular structure of chlorophyll$_a$ ($C_{55}H_{72}O_5N_4Mg$) is indicated :
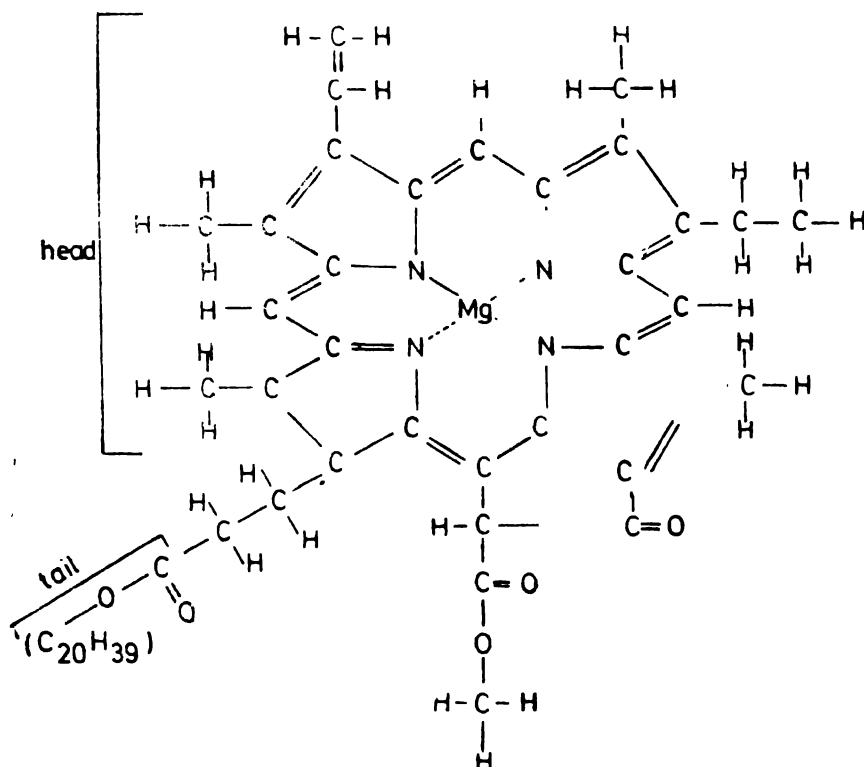
The chlorophyll sandwiches so stacked act as a battery of cells. The principal function of the chlorophyll pigments embedded in the thylakoid membrane is to absorb the light energy, which is subsequently transformed into various kinds of chemical energy utilized by the chloroplast for the reduction of CO_2 to carbohydrate. The concept of photochemical (or light) reactions in basic to an understanding of photosynthesis. The light reactions are linked to non-light-requiring enzymatic reactions (dark reactions) so as to effect the ultimate transfer of electrons from water to the acceptors that are used in the reduction of CO_2.

PHOTOLYSIS

Ordinary visible light consists of a mixture of different wavelengths, which, when unmixed, are seen in the colours of a rainbow—red, orange, yellow, green, blue, and violet. These light waves contain energy, red light has the least energy and violet the most.

On exposure to white light, the chlorophyll molecule absorbs red and violet wavelengths strongly—orange, yellow, and blue wavelengths are absorbed less strongly. The green wavelengths are hardly absorbed and are mostly reflected. This is why an illuminated leaf gives a green appearance, chlorophyll having absorbed all but the green wavelengths in light.

The chlorophyll absorbs energy through the absorption of certain light waves and in this state the chlorophyll molecule is said to be excited. Since chlorophyll has a highly conjugated structure, excitation and removal of an electron (e⁻) become rather easy. Such an electron carries most of the extra energy provided by the light and may be regarded as a high-energy electron, which is negatively charged. The chlorophyll molecule that loses the electron becomes a positively charged ion. This process of photo-ionization of chlorophyll is believed to be the fundamental energy-supplying event in photosynthesis. Chlorophyll thus appears to trap first the light energy and release some of the light-derived energy subsequently in the form of electron energy.

Some of the dislodged high-energy electrons after photo-ionization, are recaptured immediately by the same chlorophyll molecules that released them. The electrons in this process, give up their extra energy in the form of heat or light which simply dissipates. Many of the high-energy electrons are however, captured by other electron carriers in the chloroplasts and such electrons play a key role in photosynthesis where the electrons are transferred successively from carrier to the next (called cofactors) and led away from the chlorophyll molecule that released them. The electrons in such stepwise transfers, give up their extra energy progressively, and some of the released electron energy is harvested for the production of energy-rich ATP. The electron transfer can take place either in a cyclic sequence leading to ATP formation only or in a non-cyclic manner resulting in ATP formation or photophosphorylation as well as food manufacture.

One of the electron-carriers in cyclic transfer, may be ferredoxin, an iron-containing coenzyme normally present in chloroplasts. Other carriers include at least two cytochromes, coenzymes to those functioning in aerobic respiration. The high-energy electrons appear to pass from chlorophyll to ferredoxin first and then to a series of other carriers—two or more cytochromes and several others not yet completely identified. The ionized chlorophyll that lost the electrons to begin with, receive back the electrons in the end and the chlorophyll becomes electrically neutral for undergoing the process of photoionization once again, completing the cycle. The entire process can be indicated as in Fig. 8.7.
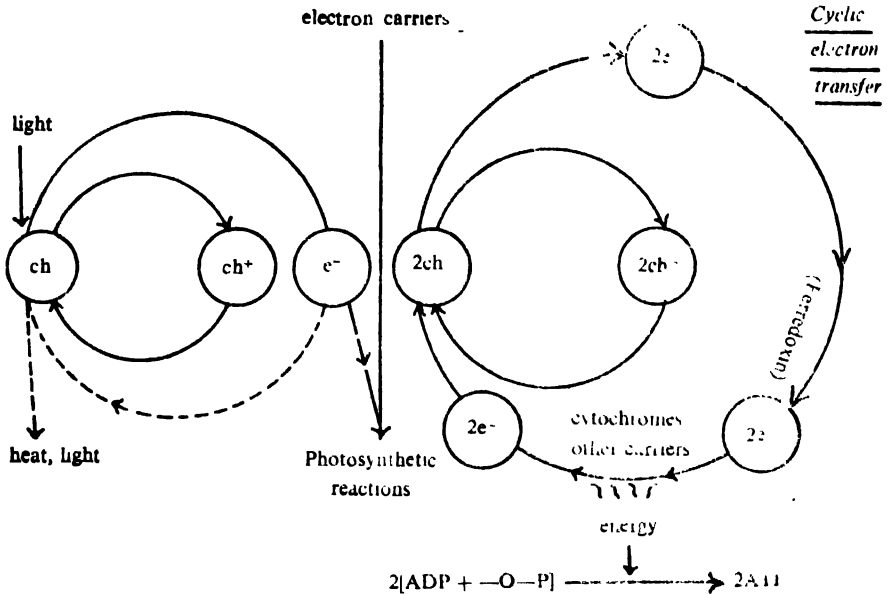
Fig. 8.7 Photoionization

PHOTOIONIZATION

High-energy electrons (e⁻) are dislodged from the chlorophyll (ch) molecules which becomes positively charged (ch⁺) on excitation by light. Some of the high-energy electrons return to chlorophyll immediately with the dissipation of extra energy as heat or light (chlorophyll becomes fluorescent). Many of the high energy electrons are captured by specific carrier molecules present in chloroplasts and take part in photosynthetic reactions.

CYCLIC ELECTRON TRANSFER

The electrons escaping chlorophyll on photoionization come back to it via carrier molecules, yielding energy for ATP formation. Two molecules of ATP are formed for every two electrons carried through the cycle. The cycle requires more energy than it yields and therefore can proceed so long as light is available for the ionization of chlorophyll, the input being light, ADP, and phosphate and the output ATP.

NON-CYCLIC ELECTRON TRANSFER

Electron carrier, ferredoxin, captures the high-energy electrons from chlorophyll. The electrons thereafter, are transferred to NADP, a hydrogen-carrying coenzyme, similar to the respiratory hydrogen-carrier

NAD. The hydrogen that NADP carries, is formed from high-energy electrons and hydrogen ions.

$$2e^- \xrightarrow{\text{NADP}} NADP\,[2e^-]$$

$$2H^+ \xrightarrow{\hspace{5cm}} NADP\,[H_2]$$
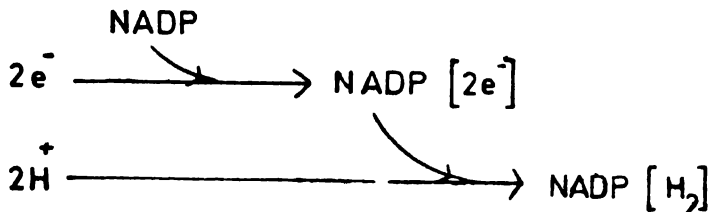
The high-enery electrons are derived from chlorophyll and hydrogen ions from water

$$2H_2 \rightleftharpoons 2H^+ + 2OH^-$$
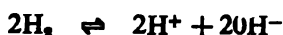
Removal of H^+ or OH^- disturbs the equilibrium resulting in ionization of more water molecules. NADP in chloroplasts, carrying high energy electrons, accepts H^+ of water. As chlorophyll is exposed to light, NADP con·inuas to receive high-energy electrons removing H^+ from water, which becomes more and more ionized. This process of light-associated decomposition of water is referred to as photolysis. It is believed that OH^- ions also play a role in photolysis by way of reorganisation to form the following:

$$H_2O$$

$$2OH^- \qquad O$$

$$2e^-$$

H_4O thus produced is available to make good the water lost in initial ionization, oxygen gas escapes as molecular oxygen accumulating in the environment as a photosynthetic byproduct, and the electrons are accepted by a pigmented carrier molecule. Two light-requiring steps appear to be involved in the non-cyclic transfer sequence. At the first step, high-energy electrons are removed from chlorophyll$_a$ which pass on to NADP without energy loss. NADP accepts H^+ from water to become NADP.$[H_2]$. The electrons derived from water and carried by a pigment are raised to a high-energy level in the second light-requiring step. These electrons return ultimately to chlorophyll$_a$ yielding extra energy in the process for ATP formation.

The chlorophyll thus loses high-energy electrons at the start and gains low-energy electrons at the end as in the cyclic sequence, the difference being that the electrons gained are not the same as those lost. Water constitutes an important intermediate compound providing electrons to chlorophyll and making possible the formation of NADP. $[H_2]$. This noncyclic process depends on the input of material from water decomposition and the input of energy from light. Two products incorporating

some of the light-derived energy result from these inputs—they are ATP in the form of high-energy phosphate bonds and NADP. [H₂], in the form of high-energy electrons in hydrogen.

Photolysis is completed with the formation of NADP.[H₂], which constitutes the immediate hydrogen source in the food manufacture and the key event in photosynthesis. There are a number of other hydrogen sources, which are however without sufficient energetic electrons.

Sunlight and water are the critical agents in the energetics of life. Hydrogen from water is energized by light and together with CO₂ becomes food and the substance of living matter through the process of photosynthesis oxygen constituting a byproduct. The food and living matter are decomposed through respiration into the original materials, CO₂ and hydrogen, and water is formed again by the union of hydrogen with oxygen. The energy gained in the decomposition reactions appears first as ATP that maintains life for a time, but dissipates ultimately as heat.

The 'light reaction' in photosynthesis indicating the cyclic and the noncyclic photophosphorylation can be presented as in Fig. 8.8.
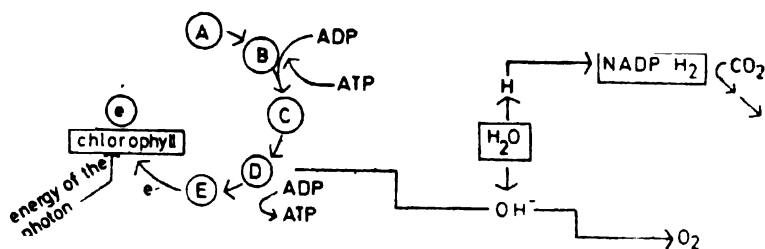
Fig. 8.8

The excitation of chlorophyll by light (energy of the photon) starts a complex series of reactions which result in the splitting of water with the liberation of molecular oxygen.

$$H_2O \qquad 2H^+ + \tfrac{1}{2}O_2 + 2e^-$$
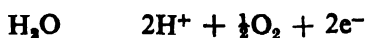
Simultaneously the hydrogen ions and electrons are used in the formation of ATP from ADP and in the reduction of NADP to NADP.H₂. The spatial arrangement of the chlorophyll and its proximal orientation to the lipids, cofactors, and protein make it active in the cell utilizing the energy of the photon.

CO₂ FIXATION

In the dark reaction carbon dioxide is fixed. The combination of CO₂ with hydrogen from NADP.H₂ is independent of light and takes place through a cyclic reaction sequence, called calvin cycle or carbon-fixation cycle. It consists of three basic steps each having several substeps catalyzed by specific enzymes. First the CO₂ enters the cycle as a raw material.

In the second step, hydrogen from $NADP.H_2$ enters as raw material. In the third step the carbohydrate end product emerges regenerating at the same time the starting point of the cycle. The starting point is ribulose 1, 5-diphosphate. One molecule of CO_2 reacts with a molecule of ribulose 1, 5-diphosphate to form two molecules of glyceric acid 3-phosphate, which are then reduced to two molecules of glyceraldehyde-3-phosphate with the help of two molecules each of ATP and $NADP.H_2$ formed in the light reaction. Six molecules of glyceraldehyde 3-phosphate are formed in this way one of which is used for conversion to glucose while the remaining five give rise to three molecules of ribulose 1, 5-diphosphate, capable of reacting with the new molecules of CO_2. This is known as the dark reaction in photosynthesis, which can be represented as:
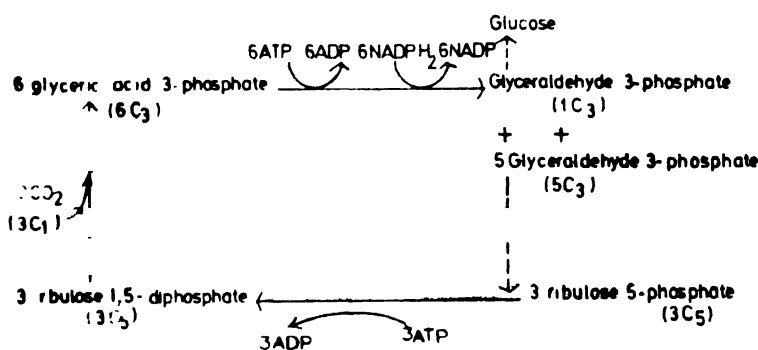
The dark and light reactions finally result in the fixation of carbon dioxide with the production of molecular oxygen and glucose. Synthesis of glucose from carbon dioxide and water is a dark reaction needing ATP and $NADP.H_2$ both of which are formed as a result of light reaction, ATP being the energy supply and the cofactor $NADP.H_2$ the reducing agent.

The two processes, photosynthesis and respiration, are complementary to each other. Photosynthesis converts inorganic oxides like CO_2 and H_2O photochemically to molecules of energy and food and releases oxygen. Respiration involves uptake of oxygen by plants and animals and oxidation of molecules of food to produce inorganic oxides and biologically useful energy. The cycle goes on through the ecosystem, or the biosphere. Green plants utilize solar energy during the day for photosynthesis—glucose synthesis takes place giving out oxygen. Photosynthesis ceases after sunset, the respiratory chain takes over consuming some glucose produced during day time and releasing carbon dioxide.

Further Reading

D.E. Green and H. Baum, *Energy and the Mitochondrion* (New York: Academic Press, 1970).

H.A. Krebs and H.L. Kornberg, *Energy Transformations in Living Matter* (Berlin: Springer, 1957).

A.L. Lehninger, *Bioenergetics* (New York: Benjamin, 1965).

J.D. Watson, *The Molecular Biology of the Gene* (New York: Benjamin, 1970).

V.G. Dethier, J.W. Lash, *et al., Topics in the Study of Life: The Biosource Book* (London: Harper Row, 1971).

C.N.R. Rao, *University General Chemistry* (Delhi: Macmillan India, 1973).

E.S. West, *et al., Textbook of Biochemistry* (Delhi: Amerind, 1974).

G.H. Bell, J.N. Davidson and D.E. Smith, *Textbook of Physiology and Biochemistry* (London: Livingstone, 1972).

P.B. Weisz, *The Science of Biology* (New York: Mcgraw-Hill, 1971).

Digestion and Absorption

Foods in general have got to be hydrolysed and chemically simplified before their assimilation in the body. This is so with carbohydrates, other than monosaccharides, and with fats and proteins. This does not happen with water or inorganic ions because they are able to pass through the digestive tract and are absorbed in their original form. Enzymes bring about the simplification of carbohydrates, fats, and proteins through a series of hydrolytic changes. These changes occur in the digestive tract which can be divided into five regions—the mouth, the esophagus, the stomach, and the small and large intestines. Secretions from the pancreas and the bile enter into the small intestine and play important roles in the digestive process. The breakdown of the naturally-occurring foodstuffs into assimilable forms is the work of digestion. The enzymes catalyse the hydrolysis of native proteins to amino acids, of the starches to monosaccharides, and of fats to glycerol and fatty acids. It is probable that in the course of these digestive reactions, the minerals and vitamins of foodstuffs are also made more assimilable. This is certainly true of the fat-soluble vitamins, which are not absorbed unless fat digestion is taking place normally.

The Mouth

The mouth contains teeth for mastication of food and salivary glands, which secrete saliva to aid in mastication. The salivary glands consist of the parotid, the submaxillary, and the sublingual, and through their ducts they pour their secretions into the mouth. The flow of saliva is regulated by a reflex stimulation of the secretory nerves. An individual may secrete about 1 to 1½ litres of saliva in the course of 24 hours.

SALIVA

Saliva is composed of approximately 99.5 per cent water, 0.2 per cent inorganic matter, and 0.3 per cent organic matter. Calcium, sodium, potassium and magnesium salts of phosphoric, hydrochloric and carbonic acids constitute the inorganic matter. The organic matter includes mucin, a glycoprotein, which gives saliva its viscous consistency. The average pH of unstimulated saliva may vary considerably on either side of neutrality, although as a rule it is slightly on the acid side, around 6.8.

SALIVARY DIGESTION

The saliva is relatively unimportant in digestion. It contains ptyalin or salivary amylase which can bring about the hydrolysis of starch to maltose. The enzyme is readily inactivated at pH 4.0 or less. Ptyalin acts on starch producing a series of ill-defined products: soluble starch, erythrodextrin, achrodextrins, and maltose. Starch and soluble starch give a blue colour with iodine, erythrodextrin gives a red colour, and achrodextrins and maltose give no colour. Maltose is the only product that reduces Benedict's solution. The optimum pH for amylase activity is 6.6. The activity of the enzyme is stimulated by the presence of halide ions, particularily chloride ion. Ptyalin becomes inactive by the removal of this ion by dialysis. Although ptyalin can bring about the hydrolysis of starch to maltose, it is actually of little significance in the body because of the short time it can act on the food. Other amylases of the intestine are capable of causing complete digestion of starch. When the food reaches the fundus part of the stomach, salivary digestion may still go on for 15 to 30 minutes, because of the slow accumulation of acid and to the partial neutralization of the acid by a temporary combination with the protein of the food.

The activity of ptyalin can be determined from the rate of starch hydrolysis. The achromatic point is the point at which iodine fails to give a colour with the substrate.

The Esophagus

The esophagus is a muscular tube connecting the mouth to the stomach. In birds and many insects there is an enlargement of the esophagus in which food is stored. This storage organ is called the crop. It is believed that the first three parts of the stomach of the ruminant are actually enlargements of the esophagus.

The Stomach

In man and the higher non-ruminant animals, the stomach is a single sac which is relatively thin-walled in the capacious fundus area, and thick-walled in the pylorus area. The inside wall of the stomach is lined with parietal (HCl-producing cells and with chief (enzyme-producing) cells. The mixed secretion of these two types of cells is called gastric juice. In ruminants, the stomach consists of fo᾿r parts, the rumen (paunch), reticulum. omasum, and abomasum. The abomasum, or fourth stomach of ruminants corresponds to the single sac stomach of man and non-ruminant animals. The gastric juice is secreted here and the digestion of proteins proceeds.

GASTRIC DIGESTION

Gastric secretion is initiated by nervous or reflex mechanisms similar to those which operate in salivary secretion. The continued secretion of gastric iuice is however dependent on a hormonal stimulus, gastrin or gastric secretin, a chemical stimulant produced by the gastric glands and absorbed into the blood which carries it back to the stomach where it excites gastric secretion. Histamine, produced by decarboxylation of the amino acids, histidine, also acts as a potent gastric secretagogue.

$$HC \overline{\quad\quad} C - CH_2 - CH - COOH \quad\quad HC \quad\quad C - CH_2 - CH_2 - NH_2$$
$$N \diagdown\diagup NH \quad\quad\quad NH_2 \quad\quad CO_2 \quad N \diagdown\diagup NH$$
$$CH \quad\quad\quad\quad\quad\quad\quad\quad\quad\quad CH$$
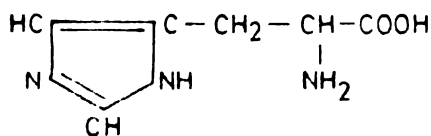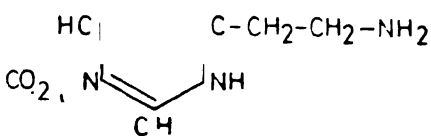
Histidine   Histamine

Digestion in the stomach involves, primarily, the action of the enzyme pepsin and hydrochloric acid on protein, producing hydrolytic products such as proteoses and peptones. The enzyme rennin occurs in the young, its action is to curdle milk. Some fat-splitting enzyme, lipase, may also be present. The food, mixed with saliva and formed into a bolus, passes through the pharynx and esophagus into the stomach, where it gradually comes in contact with the pepsin and the acid. The starch digestion however, continues in the fundus part of the stomach for sometime.

The stomach secretes about 2 to 3 litres of gastric juice in 24 hours. This secretion, which has the appearance of saliva, is a mixture of the secretions of three main types of cells: mucous, parietal and chief (zymogenic). The mucous cells secrete a liquid high in mucin, the parietal cells one high in hydrochloric acid, and the chief cells supply the proenzymes or zymogens. The parietal cells secrete hydrochloric acid solution with an acidity of approximately 0.15 N and a pH of about 1, Gastric

juice is normally a clear, pale yellow fluid of high acidity, 0.2 to 0.5 per cent HCl.

No other secretion manufactured by the body approaches the gastric juice in such high acidity. Gastric juice, like saliva, usually contains water more than 99 per cent. The material consists of mucin, the enzymes pepsin, rennin (in the young), and lipase; hydrochloric acid (0.5 per cent), and chlorides of sodium and potassium, phosphates, etc.

It is remarkable that a mineral acid like HCl acid with a concentration of 0.5 per cent, is produced in the stomach from an approximately neutral fluid. The chloride part of the acid has its origin in the chloride of the blood. In forming the acid secretion, the cells of the gastric mucosa of dogs lower the pH from 7.4 (in blood) to pH 1 to 2 (acid secretion).

In the parietal cells carbonic acid is formed. It dissociates and is catalysed in this reaction by the enzyme, carbonic anhydrase. Chloride ions pass from the plasma through the cells into the secretion. The chloride ions removed from the plasma, are replaced by the bicarbonate ions formed in the cells at the time when hydrogen ions are formed. The production of gastric HCl can be shown as in Fig. 9.1.

Plasma   Parietal cell   Lumen of stomach

$$CO_2 \qquad +CO_2 \xrightarrow{\text{Carbonicanhydrase}} H_2CO_3$$

$$H_2O'$$

$$H^{\cdot}$$

$$HCO_3^- \longleftarrow \qquad\qquad HCO_3^-$$

$$Cl^- \qquad\qquad \rightarrow Cl^{\cdot} \qquad\qquad \rightarrow Cl^-$$
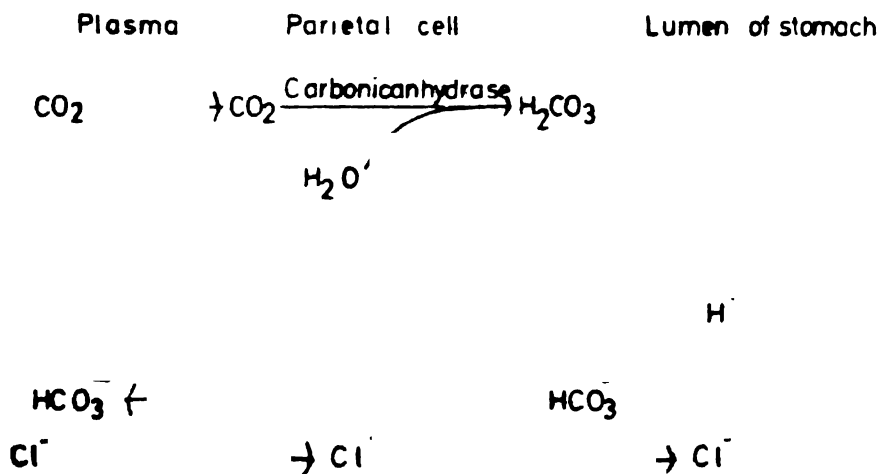
Fig. 9.1

It is believed that the reaction of fundamental importance in the production of HCl in gastric mucosa may be .

$$H_2O \qquad H^+ + CH^-$$

The H^+ ions are secreted and the OH^- ions are neutralized by CO_2 and passed into the blood.

The process is similar to that of the chloride shift in the red blood cell. After ingestion of meal, urine often becomes alkaline (alkaline tide). This may be due to the formation of extra bicarbonate in the process of hydrochloric acid secretioh by the stomach. The flow of gastric juice is

regulated by several factors such as taste, odour, sight of food (increased flow), fear or worry (suppressed flow), alcohol (increased HCl and mucin) histamine (increases HCl, decreases pepsinogen) and the hormone enterogastrone (flow decreases).

Pepsin and pensinogen

The chief digestive function of stomach is the partial digestion of protein. Gastric pepsin is produced in the chief cells is inactive zymogen, pepsinogen, which is activated to pepsin by the action c HCl and autocatalytically, by itself—a small amount of pepsin can activate the remaining pepsinogen. The enzyme hydrolyses native proteins into proteoses and peptones, which are still reasonably large protein derivatives. By incubating protein with an artificial pepsin — HCl mixture for some 24 hours, some amino acids may be produced, but this can hardly apply to gastric digestion in vivo.

The first product of peptic hydrolysis appears to be acid-metaprotein, a soluble protein which precipitates on careful addition of alkali and which coagulates when the precipitate is heated.

Proteoses and peptones are produced on further hydrolysis. Ammonium sulphate precipitates the proteoses—primary proteoses with half-saturated and secondary proteoses with the fully saturated solution of the ammonium salt. The filtrate contains the peptones, which are precipitated by some alkoloid reagents like the tannic acid. The biuret reagent gives definite violet colour with primary proteins peptones give rose-red colour.

Rennin (chymosin or rennet)

This is another enzyme elaborated by the cells of the gastric mucosa, which is probably present in relatively large quantities only in the stomach of young animals. This enzyme causes the coagulation of milk It is important in the digestive processes in infants as prevents the rapid passage of milk from the stomach. It is probable that the rennin acts on, the casein to form some soluble product, paracasein, which becomes the milk clot in presence of calcium. This enzyme is said to be absent from the stomach of adults.

Lipase

The lipolytic action of gastric juice is of little physiological importance although a gastric lipase capable of mild fat-splitting action is found in gastric juice. The important fat-splitting enzyme occurs in the pancreas.

GASTRIC ANALYSIS

Test meals are tests of gastric function, through which a study is made of the gastric secretion—of the quality and the quantity of the gastric juice in relation to its different constituents. The presence of abnormal constituents and the time taken for the meal to leave the stomach are noted. The gastric secretion is influenced by factors such as psychic, humoral and

chemical. Tests of function are based on the response to chemical and humoral stimuli. A simple meal of dry toast or oatmeal gruel or of dilute alcohol constitutes chemical stimulus and intramuscular injection of a small amount of histamine gives humoral stimulus.

Analysis of gastric juice is of importance in clinical diagnosis. It is normally about 50 ml in the fasting stomach (interdigestive period). An increase above this amount may indicate retention or regurgitation from the duodenum.

Freshly secreted gastric juice is usually colourless. Yellow or green indicates the presence of bile, due to intestinal obstruction. Red or brown colour may mean the presence of blood, which can be confirmed by the benzidine test. The blood may suggest lesions such as carcinoma of the stomach, peptic ulcer, etc.

Absence of free acid together with absence of pepsin in the gastric juice is called achlorhydria which may suggest pernicious anaemia, gastric carcinoma (cancer of stomach). Achylia denotes the absence of both HCl and the gastric enzymes.

The gastric acidity is measured in terms of ml of 0.1N NaOH required to neutralize 100 ml of gastric contents. The free HCl may be about 18.5 meaning that 18.5 ml of 0.1N NaOH are required to neutralize 100 ml of gastric juice. This is referred to as free acidity. Total acidity covers the free HCl, HCl combined with protein, acids salts (phosphates and carbonates), and organic acids such as lactic acid, butyric acid. etc. The average value of total acidity is 30 which means 30 ml of 0.1N NaOH are needed to neutralize 100 ml of gastric juice.

Different indicators are used to determine the free and total acidity of gastric juice. Topfer's reagent (dimethylamino-azobenzene) with a pH range of 2.9 to 4, is often used as an indicator in the determination of free acidity and phenolphthalein, with pH range of 8 to 9, is used for measuring total acidity.

Stomach contents are withdrawn at regular intervals after stimulation with the administration of test meal or dilute alcohol (50 ml of 7 per cent) or injection of histamine. Hypoacidity (hypochlorhydria) may indicate carcinoma of the stomach, chronic constipation, chronic gastritis (inflammation of the stomach), chronic appendicitis, etc. Gastric ulcer (peptic ulcer), duodenal ulcer, cholecystitis (inflammation of the gall-bladder), etc., may be associated with hyperacidity.

PANCREATIC DIGESTION

Food in the stomach after a time, in a thick creamy consistency called chyme, is intermittently introduced during digestion into the duodenum through the pyloric valve. The pancreatic and bile ducts open into the duodenum at a point very close to the pylorus. Here it is attacked by intestinal juice (succus entericus), pancreatic juice, and bile. The high alkaline content of the pancreatic and biliary secretions neutralize the acid

of the chyme changing its pH to the alkaline side, the shift in pH being necessary for the enzymes of the pancreatic and intestinal juice to act.

Pancreatic juice

This secretion is produced in the acinous tissue of the pancreas, and flows through the pancreatic duct, or the common bile duct into the first part of the duodenum. Pancreatic juice is a liquid like saliva, containing about 99 per cent water, 0.5 per cent inorganic matter, and 0.5 per cent organic matter. The inorganic matter is similar to those found in saliva except that $NaHCO_3$ is present in relatively high concentration, giving the pancreatic juice a pH of 7.5 to 8.0. Mucin, proenzymes trypsinogen, chymotrypsinogen, and the enzymes carboxypeptidase, pancreatic amylase and pancreatic lipase constitute the major organic matter. The flow of pancreatic juice is under both nervous and hormonal control. The walls of the duodenum contain a prohormone, prosecretin, which is converted to the hormone secretin by the acidity of the acid chyme from the stomach. Secretin enters the blood stream and stimulates the acinous tissue of the pancreas to produce pancreatic juice. Duodenal mucosa contains another hormone, pancreozymin, which increases the enzyme content of the juice without affecting the volume of the secretion. In adult man, about 800 ml of pancreatic juice are secreted in 24 hours.

Pancreatic juice is the most important of the digestive juices. It contains the enzymes which split proteins, an enzyme that hydrolyses starch, and another that hydrolyses fats. The activation of precursors (zymogens) such as trypsinogen is attributed to enterokinase, produced by the intestinal glands. A small amount of active trypsin then autocatalytically activates additional trypsinogen and chymotrypsinogen.

Protcolytic enzymes

The pancreatic juice contains the protcolytic (protein-splitting) enzymes, trypsin and chymotrypsin, which together act on native protein, proteoses, and peptones from the stomach to produce polypeptides. These two enzymes are distinguished in two ways: chymotrypsin having a much greater milk-coagulating power than trypsin and secondly the trypsinogen is activated by enterokinase to form trypsin whereas chymotrypsinogen is activated by trypsin to form chymotrypsin.

Carboxypeptidase, a zinc-containing enzyme of the pancreatic juice, and aminopeptidase and dipeptidase of the intestinal juice together bring about further breakdown of the food proteins into their constituent amino acids for absorption by the intestinal mucosa and transfer to the circulation.

Pancreatic amylase

This enzyme is similar to the ptyalin of saliva (salivary amylase), hydrolyzing starch to maltose at an optimum pH 7.1. Inositol may be a constituent of this enzyme.

Lipase

This is an important enzyme which hydrolyzes fats into fatty acid, glycerol, monoglycerides, and diglycerides. Bile salts may be necessary to activate the enzyme.

Intestines

Brunner's and Lieberkuhn's glands, embedded in the intestinal mucosa of the small intestine, secrete the intestinal juice. It contains about 99 per cent water, 0.5 per cent inorganic, and 0.5 per cent organic matter. The inorganic salts are similar to those in saliva, except that $NaHCO_3$ is present in significant amounts, giving the secretion a pH of about 8.0. Mucin, aminopeptidases, dipeptidases, enterokinase, nucleases, nucleotidases, nucleosidases, phosphatases, lactase, maltase, and sucrase constitute the major organic matter of the intestinal juice. The intestine also contains a lecithinase which hydrolyzes lecithin into fatty acid, glycerol, phosphoric acid, and choline.

    Mechanical pressure on the intestinal wall stimulates the flow of intestinal juice, it may be that the food masses provide this stimulus.

Results of Digestion

The final result of the action of the digestive enzymes is to breakdown the foodstuffs of the diet to forms which can be absorbed and assimilated. Carbohydrates breakdown to the end-products of digestion, monosaccharides, principally, glucose, proteins to amino acids, and fats to fatty acids, glycerol, and monoglycerides. Some unhydrolyzed fat is probably also absorbed.

The liver is concerned with many functions in intermediary metabolism. It also plays an important role in digestion by producing bile. The bile is stored in the gall-bladder which is attached to the liver. It accumulates in the gall-bladder during fasting and leaves the bladder to enter the small intestine during digestion, especially with fat-rich meals. In the course of digestion, the gall-bladder contracts and supplies bile to the small intestine rapidly by way of the common bile duct. The hormone cholecystokinin instigates the contraction of the gall-bladder and, probably, the relaxation of the common duct sphincter. When the gall-bladder is absent (humans with gall-bladder removal), the hepatic duct enlarges and takes over as a storage organ.

Bile

Hepatic bile is more dilute than gall-bladder bile, indicating that water is removed on storage. Gall-bladder bile contains about 86 per cent water and 14 per cent solids. The solids are made up of about 8 per cent bile salts (sodium glycocholate and sodium taurocholate), related to sterols, 2 per cent bile pigments. The difference in the composition of hepatic and gall-bladder bile is indicated in Table 9.1.

TABLE 9.1

Constituent	Hepatic bile secreted (As percentage of total bile)	Gall-bladder bile (Percentage of total bile)
Water	97.00	85.92
Solids	2.52	14.08
Bile acids	1.93	9.14
Mucin and pigments	0.53	2.98
Cholesterol	0.06	0.26
Fatty acids and fat	0.14	0.32
Inorganic salts	0.84	0.65
Specific gravity	1.01	1.04
pH	7.1-7.3	6.9-7.7

Bile has an amber colour but on exposure to r turns green, the red pigment bilirubin being converted into the green pigment biliverdin.
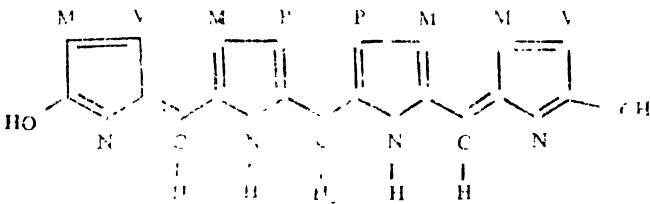
Bilirubin
$(C_{33}H_{36}O_6N_4)$

M. CH_3 (Methyl)

V CH=CH (Vinyl)

P CH_2CH_2 COOH
(Propinic acid)
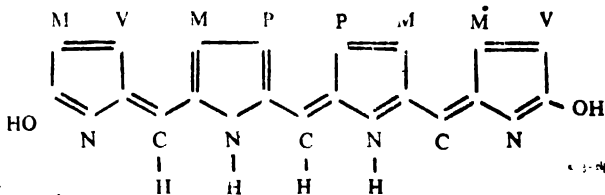
Biliverdin $(C_{33}H_{34}O_6N_4)$

Bile is alkaline in reaction and is composed of bile salts, bile pigments, lecithin, cholesterol, inorganic salts, etc. Bile is both a secretion and an excretion, the bile salts represent the secretory substances and the bile pigments—cholesterol, etc.—the excretory ones.

Bile acids

The conjugated bile acids play an important role in digestion because of their detergent and emulsifying effects on fats. Four bile acids have been isolated from human bile. They are cholic acid, deoxycholic acid, chenodeoxy cholic acid, and lithocholic acid. Cholic acid is present in the largest amount in the bile.

Deoxycholic acid does not have OH group at position 7; chenodeoxy-cholic acid having no OH group at position 12; and lithocholic acid has only OH group at position 3. Deoxycholic acid is the principal bile acid
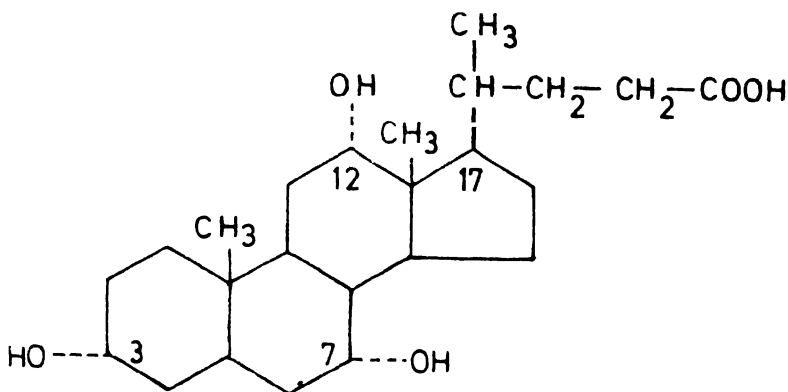
Cholic acid

present in the faeces of normal adult human beings. The bile acids are important end-products in the metobolism of cholesterol which the liver removes from the blood. Cholesterol itself, however, is also present in bile. This is a reflection of the synthesis of cholesterol by the liver. The amount of cholesterol lost from the body by removal from the blood can be measured accurately from the output of bile acids.

Bile salts

The bile acids are not excreted in the bile in the free state. They are conjugated with glycine or taurine (a cystine derivative) by the liver. The bile acids in this conjugated form are soluble in water. The conjugation with glycine or taurine takes place through the carboxyl group on the side chain of the bile acids. The conjugated bile acids are largely neutralized with sodium or potassium to form the glycocholates or

taurocholates so as to make the bile alkaline. The bile salts are made up of sodium or potassium glycocholate and sodium taurocholate. Sodium glycocholate is the sodium salt of glycocholic acid, a combination of glycine and cholic acid. Sodium taurocholate is the sodium salt of taurocholic acid, a combination of taurine and cholic acid.

$$H—CH—COOH \qquad\qquad CH_2—CH_2—SO_3$$
$$|\qquad\qquad\qquad\qquad\qquad |$$
$$NH_2 \qquad\qquad\qquad\qquad\quad NH_2$$

Glycine (Amino acetic acid)   Taurine
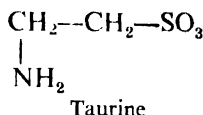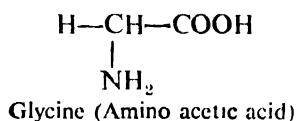
The value of bile in digestion is largely due to the bile salts which are formed in the liver. They aid in the digestion and absorption of fat and in the absorption of fat-soluble vitamins, A, D, E and K. The bile salts, mixed with fats, lower the surface tension and increase the emulsification of fats for digestion by lipase. Then the bile salts combine with fatty acids produced by the lypolytic action. A complex is formed which is more soluble and more easily absorbed. The bile salts promote the intestinal absorption of fats and fat-soluble vitamins. This is evident from the fact that excessive amounts of fat appear in the stool (steatorrhea) when bile is excluded from the intestine.

Cholic acid resembles cholesterol in structure, the side-chain in these two compounds being somewhat different. The conjugation of cholic acid requires preliminary activation with Coenzyme A. The activating enzyme occurring only in the microsomes of the liver, catalyses the conjugation. The adult human secretes about one litre of bile in 24 hours. A large proportion of the bile salts excreted into the intestines via the bile duct is reabsorbed and returned to the bile by the liver. The presence of bile salts in the intestines is the main stimulus to bile secretion by the liver.

Bile pigments

The bile pigments originate mostly from the decomposition of haemoglobin of the red cell. Bilirubin is the major bile pigment; it is orange in colour and is a partially reduced form of biliverdin. Reduction of bilirubin gives colourless meso-bilirubinogen:

Sterco-bilinogen is believed to be formed by the action of intestinal bacteria on meso-bilirubinogen. Both of them are excreted in the faeces and urine. Autoxidation of both of them results in the formation of stercobilin and urobilin. Stercobilinogen and stercobilin are excreted in the largest amount. Some of the bile pigments are reabsorbed into the circulation where most of it is re-excreted via the bile into the intestinal tract. Bilirubin in combination with the α-globulin fraction, occurs in the plasma. The combined bilinogens in the urine are referred to as the urobilinogen.
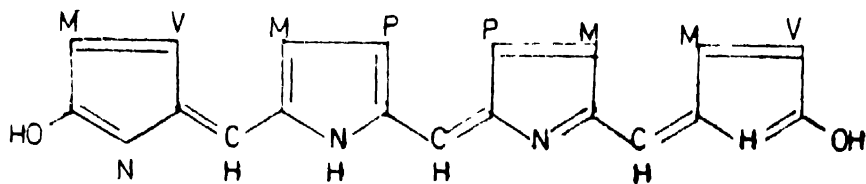
Biliverdin
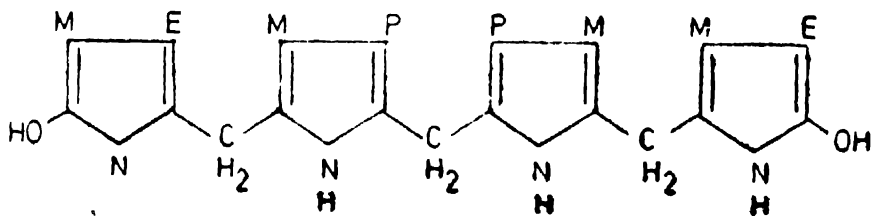
Mesobilirubinogen
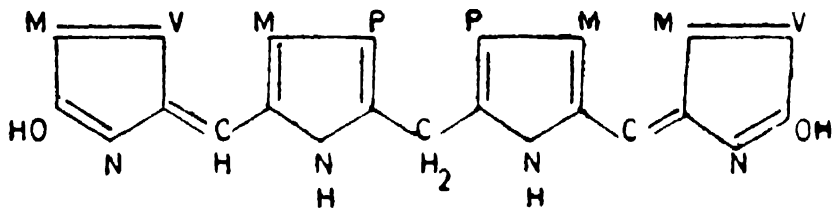
Bilirubin

M   methyl (CH₃)

V   vinyl (CH=CH₂)

P   propionic acid (CH₂ CH₂COOH)

E   ethyl (CH₂ CH₃)
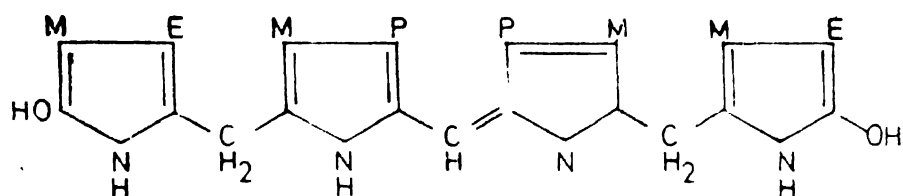
Urobilin
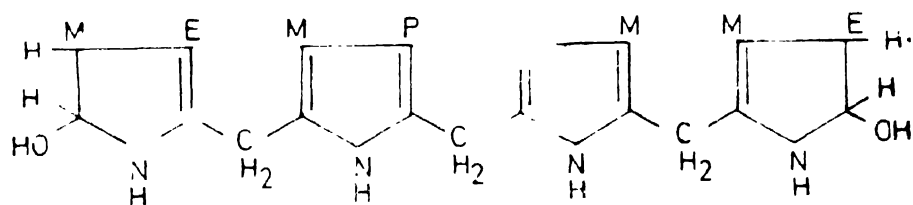
Stercobilinogen
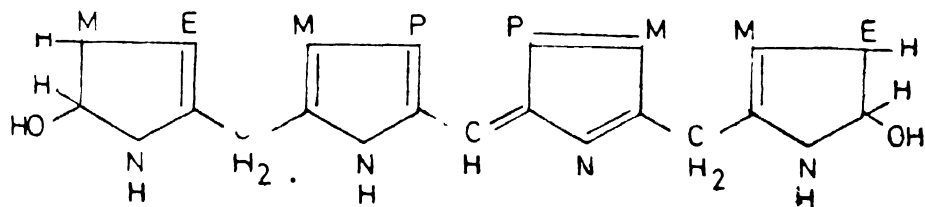
Stercobilin

The bile pigments on oxidation with nitric acid give a series of coloured products; the test is known as Gmelin's Test. Bile salts give red colour when treated with sucrose and concentrated sulphuric acid (Pettenkofer's test).

Gall-stones

In pathological conditions gall-stones (biliary calculi) are formed in the bile ducts and in the gall-bladder, and they may prevent the bile from entering the intestines. Although these stones may contain some calcium, they are usually made of cholesterol with the admixture of some bile pigment. Occasionally they consist mainly of bile pigment, cholesterol mixed with the calcium salts of bilirubin, carbonate, or phosphate.

The calculi in the gall-bladder and the bile ducts (cholelithiasis) are generally associated with inflammation of the gall-bladder (cholecystitis) and of bile passages (cholangitis). Formation of pure gall-stones probably takes place when the gall-bladder is unable to handle the cholesterol reaching it. Infection and inflammation of the gall-bladder often give rise to mixed gall-stones. X-ray examination of the gall-bladder after administration of tetra-iodophenolphthalein (radio opaque compound) is often of use in the diagnosis of gall-bladder disease.

Jaundice (Icterus)

Jaundice is caused when an excess of bile pigments gets into the blood, the skin and secretions turning yellow in colour. Complete or partial obstruction of the common duct leads to the common form of the disease, obstructive jaundice. Haemolytic jaundice, another form of the disease, is caused by the extensive destruction of the haemoglobin. Tests for bile pigments and bile salts in the urine; urobilinogen in urine; and the van den Bergh reaction and icterus index for bilirubin in plasma or serum are frequently used to distinguish obstructive from haemolytic jaundice. In obstructive jaundice the bilirubin has passed through the liver whereas it has not passed through the liver in haemolytic jaundice.

Putrefaction

The small intestine is concerned with most of the absorption of food-stuffs. The unabsorbed foodstuffs pass on to the large intestine, where water is gradually lost by absorption and the products are finally eliminated as faeces. The normal stool consists of a mixture of water; undigested food; products of the digestive tract such as bile pigments, enzymes, mucus; products of putrefaction like indole, skatole, fatty acids, gases, etc.; epithelial cells from the walls of the intestine; bacteria, etc. Active bacterial action taking place in the large intestine results in the formation of gases, hydrogen, carbon dioxide, ammonia, hydrogen sulphide, methane; acids, acetic, lactic, butyric; various toxic substances, like indole, skatole,

phenol, etc. The bacterial decomposition of carbohydrates largely accounts for the production of the acids. Lecithin gives rise to some special products like choline, neurine, and muscarine:

$$CH_3 \diagdown \qquad \diagup CH_2CH_2OH$$
$$CH_3 \longrightarrow N^+$$
$$CH_3 \diagup$$

Choline

$$CH_3 \diagdown \qquad \diagup CH=CH_2$$
$$CH_3 \longrightarrow N^+$$
$$CH_3 \diagup$$

Neurine

$$CH_3 \diagdown \qquad \diagup CH_2 \, CHO$$
$$CH_3 \longrightarrow N^+$$

$$CH_3$$
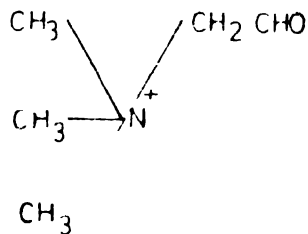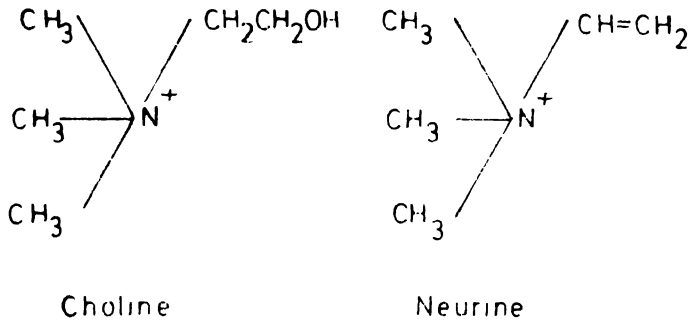
Muscarine

The proteins after preliminary hydrolysis into their respective amino acids, are involved in deamination and decarboxylic reactions leading to the most characteristic group of products.

$$\underset{NH_2}{R.CH.COOH} \xrightarrow[-NH_3]{\text{deamination}} \underset{\text{(Fatty acid)}}{R.CH_2.COOH}$$

$$\underset{NH_2}{R.CH.COOH} \xrightarrow[-CO_2]{\text{decarboxylation}} \underset{\underset{\text{(Amine)}}{NH_2}}{R-CH_2}$$
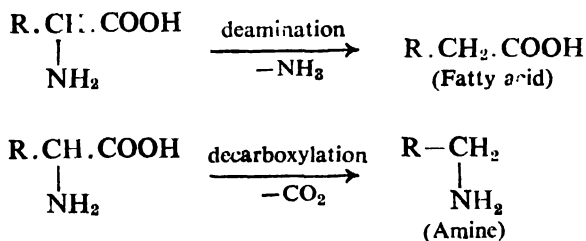
The amino acid : yptophan, gives rise to indole and skatole—the products responsible for the odour of faeces:

Tryptophen

Indole ethylamine

Indolephropionic acid

Indole

Indoleacetic acid

Skatole
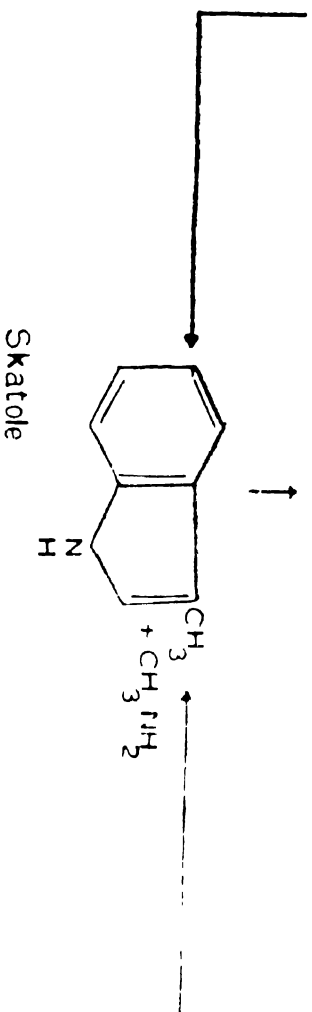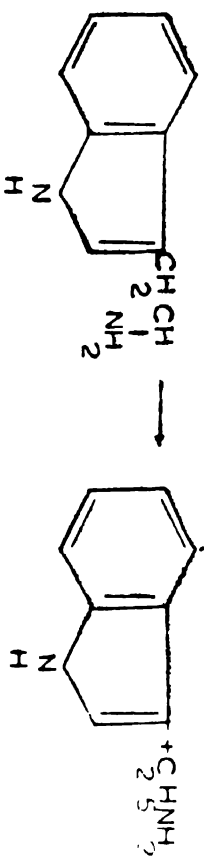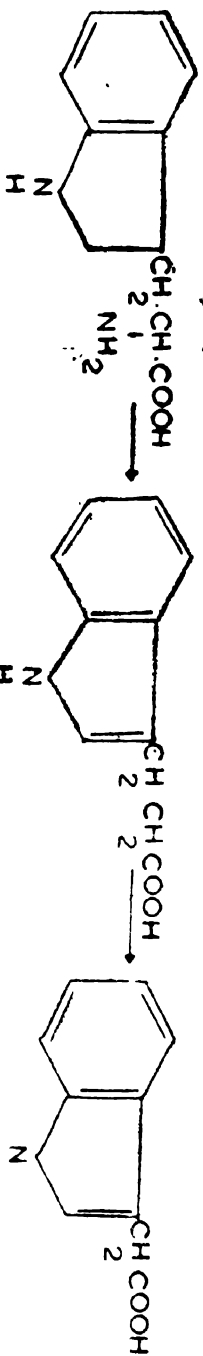
Sulphur-containing amino acid cystine yields marcaptans:

$$CH_2-S-S-CH_2$$
$$CHNH_2 \quad CH.NH_2 \longrightarrow CHNH_2 \longrightarrow CH_2 \longrightarrow CH_2$$
$$COOH \qquad COOH \qquad COOH \qquad COOH$$

Cystine / Cysteine / Thiopropionic acid / Ethylmercaptan

Structures with CH$_2$SH, CH$_2$SH, CH$_2$SH labels.

$$CH_2SH$$
$$CH_2NH_2 \longrightarrow CH_3SH + CH_3NH_2$$

Aminoethyl mercaptan / Methyl mercaptan

$$CH_4 + H_2S$$
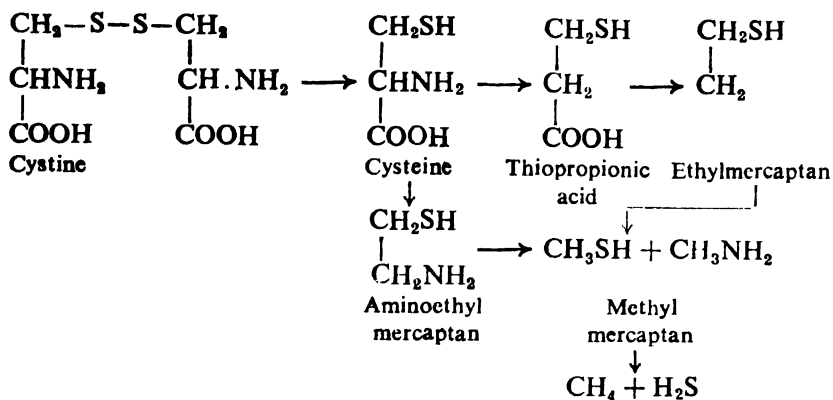
Decarboxylation of lysine and arginine gives rise to cadaverine and putrescine respectively, which constitute the so-called ptomaines obtained from putrefying flesh:

$$CH_2 \, CH_2 \, CH_2.CH_2.CH.COOH \longrightarrow CH_2.CH_2.CH_2.CH_2.CH_2$$
$$NH_2 \qquad\qquad NH_2 \qquad\qquad NH_2 \qquad\qquad NH_2$$
Lysine / Cadaverine

$$HN=C \underset{N.(CH_2)_3.CH.COOH}{\overset{NH_2}{\big\langle}}$$
$$\qquad\qquad H \qquad NH_2$$
OH / H
Arginine

$$O=C\overset{NH_2}{\underset{NH_2}{\big\langle}} \quad + \quad CH_2.(CH_2)_2.CH \, COOH$$
Urea $\qquad\qquad\qquad$ NH$_2$ $\qquad$ NH$_2$

$$Ornithine \longrightarrow CH_2 \, (CH_2)_2 \, CH_2$$
$$\qquad\qquad\qquad NH_2 \qquad\qquad NH_2$$
Putrescine
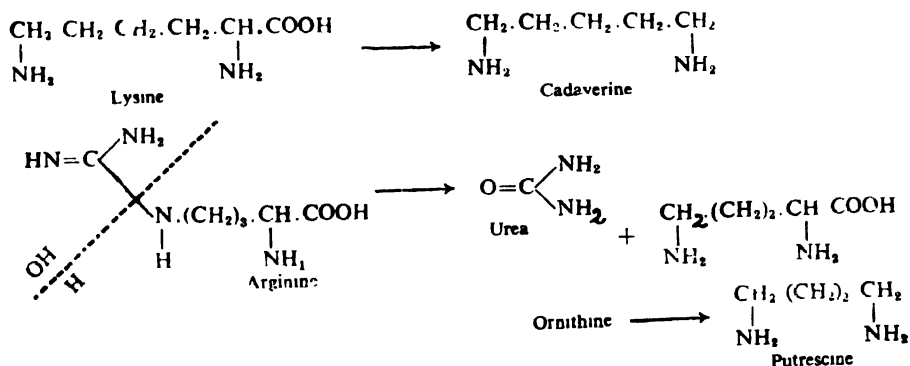
Decarboxylation of histidine yields a highly toxic substance histamine (may be identical with the gastrin of the stomach); the allergic reactions may be due to this:

$$HC = C.CH_2CH.COOH \qquad\qquad HC = C.CH_2.CH_2.NH_2$$
$$HN \quad N \quad NH_2 \longrightarrow HN \quad N$$
$$CH \qquad\qquad\qquad\qquad CH$$
Histidine $\qquad\qquad\qquad\qquad$ Histamine
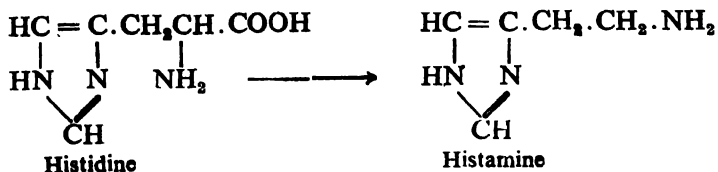
TABLE 9.2

THE SUMMARY OF THE DIGESTIVE PROCESSES

Source of enzyme and stimulus for secretion	Enzyme	Activation method and optimal condition	Substrate	End product
1. Salivary glands: Reflex response to presence of food in mouth produces secretion of saliva	Salivary amylase	Activation needs chloride ion pH: 6 6·6.8	Starches	Maltose
2. Stomach glands: Gastric juice is secreted by the chief and parietal cells due to reflex stimulation and chemical action of gastrin.	Pepsin	Conversion of pepsinogen to active pepsin by HCL pH: 1.0-2.0	Protein	Proteoses and peptones
	Rennin	Activation needs calcium pH: 4.0	Casein of milk	Milk coagulation
3. Pancreas: Duodenum is activated by the acid chyme from stomach to produce:	Trypsin	Conversion of trypsinogen to active trypsin by enterokinase of intestine pH: 5.2-6.0	Protein, proteoses, peptones	Polypeptides Dipeptides
(i) Secretin stimulating hormonally the flow of pancreatic juice.	Chymotrypsin	Autocatalysis at pH 7.9 Conversion of chymotryp sinogen to active trypsin pH: 8.0	Protein, proteoses, peptones	Polypeptides Dipeptides Increased milk coagulation
	Carboxypepti-dase	Secreted as proenzyme, activated by trypsin	Polypeptides having free carboxylic groups	Lower peptides Free amino acids
	Amylase (amy-lopsin)	pH: 7.1	Starch	Maltose
	Lipase (steapsin)	Bile salts may be responsible for activation pH: 8.0	Primary ester linkages of fats	Fatty acids, mono and diglycerides, glycerol

	Secretion/Enzyme	Conditions	Substrate	Products
	Ribonuclease		Ribonucleic acid	Nucleotides
	Deoxyribonuclease		Deoxyribonucleic acid	Nucleotides
	Cholesterol esterase	Bile salts needed for activation	Esters of cholesterol with fatty acids	Free cholesterol
4. Liver and gall bladder	Bile salts and alkali	Hormones, cholecystokinin and hepatocrinin from intestine stimulate gall-bladder and liver to secrete bile.	Fats: Acid chyme is also neutralized	Fatty acids, bile salts conjugate and emulsify neutral fat
5. Small intestine: Flow of succus entericus induced by enterocrinin.	Aminopeptidase		Polypeptides with free amino groups	Lower peptides, free amino acids
	Dipeptidases	pH: 5 0-7 0	Dipeptides	Amino acids
	Sucrase	pH: 5.8-6 2	Sucrose	Fructose, glucose
	Maltase	pH: 5.4-6 0	Maltose	Glucose
	Lactase	pH: 8.6	Lactose	Glucose, galactose
	Phosphatase		Organic phosphates	Free phosphate
	Isomaltase or 1:6 glucosidase		1,6 glucosides	Glucose
	Polynucleotidase		Nucleic acid	Nucleotides
	Nucleosidases		Purine or pyrimidine nucleosides	Purine or pyrimidine bases, pentose phosphate
	Lecithinase		Lecithin	Glycerol; fatty acids; phosphoric acid; choline

Tyrosine gives rise to tyramine which is in a way similar to epinephrine in raising blood pressure.

The summary of the digestive processes is indicated in Table 9.2.

Absorption

The absorption of foodstuffs in the mouth and esophagus is not appreciable although some drugs such as trinitrin, morphine and steroid hormones, are absorbed through the oral mucous membrane. Absorption through the gastric mucosa is very limited although small amounts of water, undissociated organic acids such as acetyl salicylic acid, and alcohol may be absorbed.

The site of absorption in the small intestine is determined by the relationship between the rate of transit and the rate of absorption and also as to whether the substance is transferred by an active transport mechanism or by diffusion. Substances are absorbed through the intestinal mucosa against a concentration gradient under active transport mechanism. Metabolic inhibitors reduce the rate of active transfer because the processes require energy. The absorption due to active transport is usually rapid and therefore takes place in the jejunum. Diffusion through the intestinal mucosa in the same direction as the concentration gradient results in passive absorption. Metabolic energy is not required in the process of diffusion because the rate of transfer is not affected by the metabolic inhibitors. The rate of transit through the intestine and the luminal concentration of the substance to be transferred determine the site of absorption when it is passive.

A specialized carrier mechanism is required for substances transported actively and the site of absorption therefore is the site of the carrier. Vitamin B_{12} and bile salts are absorbed for this reason in the terminal ileum. Absorption of water and food materials takes place most actively in the upper part of the small intestine. The absorption of foodstuffs in health, is almost complete during passage through the small intestine, all of the carbohydrate, about 95 per cent of fat and 90 per cent of the protein being absorbed. Digestion and the gut movements prepare the food for absorption.

SODIUM ABSORPTION

The gut wall is very permeable to sodium and there are large passive movements in both directions. Charged particles move towards charge of the opposite sign. Movements which occur in accordance with such electrochemical gradients do not require metabolic energy and are said to be passive. The electrochemical gradient will determine whether the net movement of sodium is into or out of the gut lumen. Superimposed upon these passive movements is an active absorption as demonstrated in length

of intestine bathed in oxygenated fluids in vitro.

Sodium can move against an electrochemical gradient from mucosal side to serosal side. This ability is abolished by the withdrawal of oxygen or glucose from the bathing fluid. This movement against the gradient is also eliminated by metabolic inhibitors such as cyanide.

The active process cannot remove all the sodium from the lumen. The epithelium is so permeable that when the luminal sodium concentration is low, the passive flux into the lumen is equal to the sum of the active and passive fluxes out of the lumen. The limiting luminal concentration is about 65 meq per litre. (The plasma sodium concentration is about 130 meq per litre.) Sodium is absorbed at luminal concentration above this and at concentrations below it passes into the lumen.

CHLORIDE ABSORPTION

Much the same considerations govern chloride absorption. Chloride absorption is believed to be usually active. It has been claimed that, as in the kidney, the absorption of the positively charged sodium ions drags along the negative chloride ions passively. The experimental evidence is however inconclusive.

OTHER MONOVALENT IONS

Potassium is probably absorbed passively. Bicarbonate is effectively absorbed but the details are obscure. Bicarbonate is secreted in many digestive juices and is probably manufactured in the mucosal cells. It may be destroyed by contact with gastric acid which converts it into carbon dioxide and water. Iodide is secreted into the gut in the saliva and other secretions but most is rapidly absorbed.

POLYVALENT IONS

On the whole, the gut is less permeable to polyvalent ions and serious deficiency may occur because of poor absorption.

Calcium
Like sodium calcium is absorbed both actively and passively. Vitamin D is essential for the active process and also influences the passive process by increasing gut permeability.

Iron
The daily requirement in adult men is about 1 mg and in women about 2 mg because of the menstrual loss. Although the daily intake of iron ranges between 10 to 20 mg, poor absorption may lead to precarious iron balance. Ferrous iron is more permeable to the gut than the ferric iron, which may be due to the large size of the hydrated trivalent ion. The

acidity of the gastric juice promotes the reduction of ferric to ferrous ion. Serious iron deficiency anaemia may occur after the removal of stomach or when abnormally small amount of acid is secreted. The absorption of iron is partly active and partly passive and depends on the state of the body's iron stores.

Phosphate

This is absorbed partly as HPO_4^{--} and partly as $H_2PO_4^{-}$. Some may be absorbed actively.

Water absorption

It has been assumed by most workers that water absorption must be passive. The problem is enormous, since in a normal adult, 7 to 8 litres of water are absorbed from the gut each day. Much of this comes, of course, from the gut secretions. There are three ways by which water could be moved passively: (1) By a hydrolytic pressure difference between the gut lumen and the blood. (2) By ill-defined molecular forces such as the dragging along of hydration water when hydrated ions are actively absorbed. (3) By osmotic forces.

The last is by far the most important, the first two being of little practical significance. Relatively little osmotic equilibration takes place in the stomach and the fluid which reaches the duodenum may often be hypertonic or hypotonic to plasma. In the small intestine, the initial event is an equilibration which may involve the movement of water into or out of the gut. Once osmotic equilibrium has been reached, it may be upset by two factors: (1) The digestion of large fat, protein, and carbohydrate molecules to smaller units raises tonicity. (2) The active absorption of ions and molecules reduces tonicity.

The second process, in fact, outstrips the first and the net effect is the reduction in the number of osmotically active particles in the gut lumen. This leaves the lumen hypotonic to plasma and so water follows the particles out of the lumen in order to maintain the osmotic balance.

Thus the active absorption of dissolved particles determines water absorption. Water absorption is, however, essential for both the active and passive absorption of most of the dissolved material. Sodium could, for example, be considered. The active mechanism can set up a concentration difference of about 65 meq per litre between the plasma and gut lumen. With isotonic saline solution placed in the intestine, if water did not follow the sodium, the limiting gradient would be set up when only half the sodium had been absorbed. However, if water follows, the sodium concentration in the lumen will remain close to that in the plasma. Most of the sodium will be absorbed because the concentration gradient never approaches its limiting value.

Water movement is also important in passive absorption, as evident from potassium ion. At the upper end of the small intestine, passive electrochemical gradients will ensure that the luminal concentration

approaches the plasma concentration. Whether this means absorption or movement from blood to lumen will depend on the potassium content of the meal. Once this equilibrium has occurred, the reabsorption of water will raise the potassium concentration in the intestine and potassium will therefore move into the plasma. This will continue until most of the water and most of the potassium have been absorbed. The passive absorption of water and of many other substances depends on the active absorption of sodium and other ions and molecules.

INTESTINAL FLORA

The intestinal flora may comprise as much as 25 per cent of the dry weight of the faeces and probably 25 per cent of the dried faeces represents bacteria, mostly non-pathogenic. Much of the food is utilized in hervivora by the bacterial decomposition of whatever foodstuffs remain in the large intestine. This bacterial decomposition of diet consisting largely of cellulose, is of particular importance to hervivora, since the intestinal or ruminal bacteria are essential to digestion as they decompose the polysaccharide and make it available for absorption. These symbiotic bacteria, in addition, may be concerned with the synthesis of essential amino acids for these animals.

The intestinal flora in man is not that important but still the bacterial activity play a role in providing some nutritional benefit to man as evident from the synthesis of certain vitamins, particularly vitamin K, and possibly some members of the B complex, which are made available to the body.

The colon bacillus is the commonest organism found in man, concerned with the production of putrefactive toxic substances. Another organism belonging to the aciduric group, is present in much smaller quantity. Lactic acid is produced from carbohydrate by such bacteria establishing a medium which is unfavourable to colon bacillus.

The intestinal flora plays in general, an important role in nutrition. Some of these micro-organisms are capable of synthesizing a number of vitamins, some amino acids, and possibly even some fatty acids and the needs for these nutrients are met, to some degree, from the microflora.

Foods are required to be converted into simple, soluble form before being absorbed. The digestive juices bring about the simplification of foodstuffs into amino acids, hexoses (glucose, fructose and galactose), glycerol and fatty acids. Pentoses and mannose may be formed if pentosans and mannosans are present. Animal nucleic acid may also form some pentose.

Absorption through the stomach wall is generally slight. The absorption of food including much of the water occurs most readily through the walls of the small intestine. The length of the tube is about 25 feet and its surface area is considerably increased by the villi. It has been shown that 90 per cent of the protein is absorbed here so also the carbohydrates and fats. Water also is absorbed here but its loss appears

to be made up by diffusion of liquid into the intestine, for the food at the ileocecal valve is still very fluid.

The absorption of much of the water in the large intestine results in a residue which eventually is excreted as faeces.   The bacterial action leading to the putrefactive process, is very pronounced here.

ABSORPTION OF CARBOHYDRATES

Carbohydrates are absorbed from the intestine into the blood of the portal venous system in the form of monosaccharides, through the capillaries of the villi.   The monosaccharides are chiefly hexoses—glucose, fructose, galactose and mannose.   Pentose sugars, if present in the food ingested, are also absorbed, though more slowly than the hexoses.   The rate of absorption of the hexoses differs indicating a selective action on the part of intestinal mucosa in the order: galactose, glucose, fructose and mannose.   The passage of hexose across the intestinal barriers occurs at a fixed rate, even against an osmotic gradient and is not affected by its concentration in the blood.   The process of hexose absorption must therefore involve a force other than that of simple diffusion.

It has been assumed that the source of such energy for the process of active absorption is provided by the mechanism of phosphorylation of the hexoses as a necessary preliminary, to absorption.   This is evident from the fact that the hexosephosphate content of the intestinal mucosa increases during the absorption of sugars and also from the fact that the two substances, monoidoacetic acid and phlorhizin, which prevent such phosphates formation by inhibiting the phosphorylating enzymes, prevent the absorption of sugars from the small intestine.   The metabolic inhibitor, phlorhizin specifically interferes with active glucose transport but not with the transport of other actively absorbed materials.   It appears to interfere with the movement of glucose into the epithelial cells.

The hexoses are absorbed at rapid rates only through the living membrane and the rates of absorption of different hexoses against that of hexose taken as 100, are as below (data taken from Cori, using rat as experimental animal):

Galactose	= 110	Mannose	= 19
Glucose	= 100	Xylose	= 15
Fructose	= 43	Arabinose	= 9

The hexoses are converted largely into glycogen in the liver and stored there as such until needed by the body.

ABSORPTION OF LIPIDS

Two opposite theories for the absorption of lipids have been postulated.

According to one theory all fat must be split to fatty acids and glycerol before absorption and the other held that an appreciable amount of fat as such can be absorbed, provided it is at first thorougly emulsified by bile. It has been shown that the digestion of fats yields mono and diglycerides and free fatty acids, with the release of only small amounts of free glycerol.

The 2-monoglycerides are less stable than the 1-monoglycerides and are converted to the latter in the dige tive mixture. The fats can be hydrolyzed by the lipase in the intestine into glycerol and fatty acids. The glycerol is soluble and is easily absorbed.

$$
\begin{array}{lll}
& \overset{\overset{\displaystyle O}{\parallel}}{CH_2-O-C-R} & \qquad \overset{\overset{\displaystyle O}{\parallel}}{CH_2-O-C-R}\ \ CH_2OH \\[3mm]
\overset{\overset{\displaystyle O}{\parallel}}{CH-O-C-R} \xrightarrow[H_2O]{lipase} & CHOH \quad + \quad \overset{\overset{\displaystyle O}{\parallel}}{CH-O-C-R} + \overset{\overset{\displaystyle O}{\parallel}}{R-C-OH} \\[1mm]
& & \qquad\qquad\qquad\qquad\qquad \text{Fatty acid} \\[3mm]
\overset{\overset{\displaystyle O}{\parallel}}{CH_2-O-C-R} & \overset{\overset{\displaystyle O}{\parallel}}{CH_2O-C-R} \quad \overset{\overset{\displaystyle O}{\parallel}}{CH_2-O-C-R} \\
\quad \text{Triglyceride} & \text{1,3 diglyceride} \quad\ \ \text{1,2 diglyceride}
\end{array}
$$

$$
\xrightarrow[H_2O]{lipase} \ \
\begin{array}{l}
CH_2OH \\
| \\
CHOH \\
| \\
CH_2-O-\overset{\overset{\displaystyle O}{\parallel}}{C}-R \\
\text{1-monoglyceride}
\end{array}
\quad + \quad
\begin{array}{l}
CH_2OH \quad \overset{\overset{\displaystyle O}{\parallel}}{\ } \\
| \\
CH-O-\overset{\overset{\displaystyle O}{\parallel}}{C}-R \\
| \\
CH_2OH \\
\text{2-monoglyceride}
\end{array}
\quad + \quad \overset{\overset{\displaystyle O}{\parallel}}{R-C-OH} \\
\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{Fatty acid}
$$
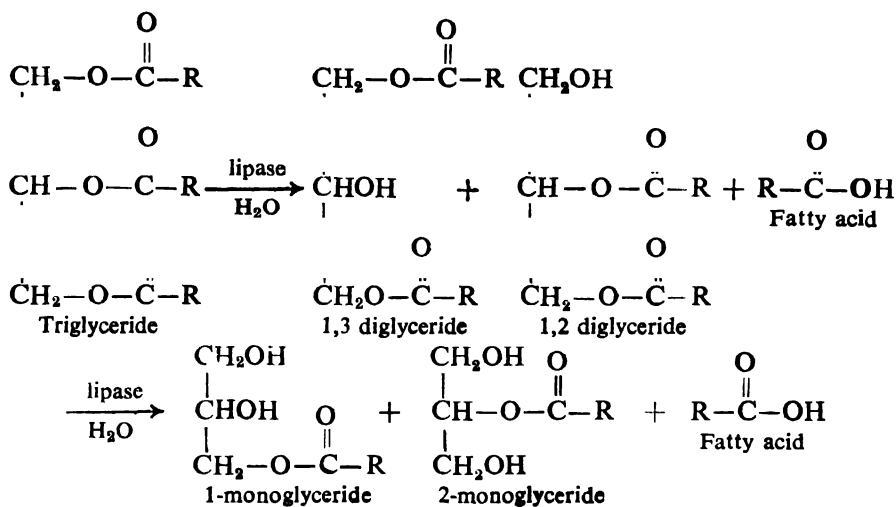
Mono and diglycerides in conjunction with bile emulsify undigested triglycerides to a particle size of 0.5 microns or les , which have been named chylomicrons capable of being absorbed directly through the intestinal epithelium. The hydrolysis of triglycerides stops at the stage of di- and monoglycerides and the digestion mixture consists of free fatty acids, di- and monoglycerides as well as unsplit (or resynthesized) triglycerides, the monoglycerides accounting for as high as 50 per cent of lipids in the human intestinal contents after a fat meal. Calcium ions increase the proportic 1 of monoglycerides during fat digestion by removing fatty acids from the interface between the oil and water phases of the digestion mixture. Lipases have been found to be presen in gastric juice, pancreatic juice, and intestinal juice. It is believed that the only lipase that plays a significant role in the digestion of fats is pancreatic lipase having its optimum activity at pH range 7.5 to 8, and is a potent tri- and diglyceridase.

Free fatty acids, mono- and diglycerides, and chylomicrons (triglyceride globules with diameters of 0.5 microns or less) pass through the epithelium of the villi. As these hydrolysed and unhydrolysed fragments pass through (1) the outer border, (2) the body, (3) the basal membrane of

the mucosal epithelial cells of the villi, the free fatty acids are converted to triglycerides. In this resynthesis, the free fatty acids combine with either simultaneously absorbed mono- or diglycerides, or with endogenous glycerol precursor, which may be dihydroxy acetone. Free dietary glycerol does not participate in this resynthesis as shown by tracer studies. Combination with phosphoric acid (phosphorylation) is perhaps a preliminary to actual resynthesis. However, phospholipids (lecithin) are not intermediates in the passage of fats through the intestinal wall. The resynthesized fat is not quite the same as the original dietary fat.

The chilomicrons, performed or synthesized in the wall of the villi, are transferred mainly to the lacteal, where they enter the lymph and finally into the venous circulation via the thoracic duct. A small proportion enter the blood capillary system of the villi. The fat which finally appears in the blood is either stored in adipose tissue, etc., or metabolized.

The utilization of fatty acids for resynthesis of triglycerides requires activation involving the formation of a coenzyme A (acyl) derivative of the fatty acid. The reaction requiring ATP, is catalysed by the enzyme thiokinase:

$$R-COOH \xrightarrow[\substack{Mg^{++} \\ ATP \qquad AMP+PP}]{\text{Thiokinase}} R-\overset{\overset{\displaystyle O}{\|}}{C} \sim S-CoA$$
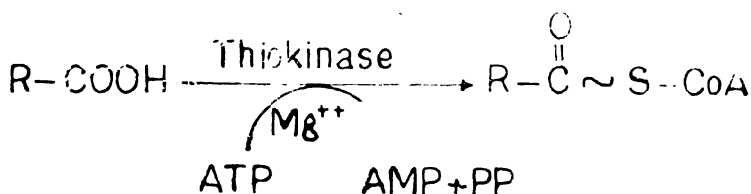
An ATP-dependent fatty acid thiokinase has been found to be present in the mucosal cells of the intestine.

The free glycerol released in the intestinal lumen is not reutilized but passes directly to the portal vein. The glycerol released however, within the intestinal wall cells can be reutilized for triglyceride synthesis.

The majority of the absorbed fatty acids of more than ten carbon atoms in length, are found as esterified fatty acids in the lympth of the thoracic duct. Fatty acids of less than ten carbon atoms in length, are transported in the portal venous blood as unesterified (free) fatty acids. These lower fatty acids are not important constituents of fats ordinarily taken in the diet.

Chyluria or excretion of milky urine is an abnormality that occurs due to 'the presence of an abnormal connection between the urinary tract and the lymphatic drainage system of the intestine. Fat absorption is seriously impaired by a lack of bile in the intestine as may occur when the bile duct is completely obstructed.

ABSORPTION OF LECITHIN

Lecithin and phospholipids are believed to be hydrolysed in the small

intestine before any absorption can take place. The intestinal mucosa and pancreatic juice, among others, contain the phospholipids—the splitting enzymes. Pancreatic juice contains an enzyme which brings about partial hydrolysis of lecithins and cephalins, liberating fatty acids and this enzyme is believed not to be identical with lipase. A part of the phospholipid is split in the small intestine with the removal of phosphate or glycerophosphate, which is absorbed as such and a portion can be absorbed as intact molecule as shown by studies using labelled phospholipids.

CHOLESTEROL ABSORPTION

The amount of fat or the kind of fatty acids absorbed determines to some extent, the absorption of cholesterol. The solubility of cholesterol in bile may be a factor in its absorption. Cholesterol is absorbed through the lecteals accompanied by the esterification of the sterol. Cholesterol both free and esterified (cholesterol with fatty acids) is found in blood. This has led Bloor to suggest that cholesterol may function as a transporter of fatty acids. Cholesterol is absorbed by the animal, which cannot do so with the sterol of the plant kingdom, sitosterol. Ergosterol is also not absorbed unless it is irradiated—when it becomes a vitamin D.

ABSORPTION OF PROTEINS

Infants, mammals of many species can absorb intact protein from the gut. This may be useful in the passive transfer of antibodies from maternal milk. The capacity of the human infant in this respect is very limited. The gut of most adult mammals is virtually impermeable to undigested protein. Under normal conditions dietary proteins are almost completely digested to their constituent amino acids and these end-products of protein digestion are then absorbed from the intestine into the portal blood. Blood always contains amino acids and the amino acids content in blood indicate definite increase after a meal rich in proteins. Animals can be maintained in a satisfactory nutritional state with respect to protein when fed on a complete amino acid mixture, indicating that intact protein is not necessary.

There is a difference in the rate of absorption of various L- and D-isomers of amino acids from the intestine. The natural (L) isomer is actively transported across the intenstine from the mucosa to the serosa, vitamin B$_6$ (pyridoxal phosphate) being involved in the transfer. The D-isomers are however transported only by free diffusion. The rates of diffusion of both isomers are the same. A diffusion process alone cannot explain the difference in the rates of absorption of the two isomers. The active transport of L-amino acids is energy-dependent, ATP being the energy source for active transport.

Most of the amino acids have active transport systems. They can be

divided into three main groups. All the members of one group compete with one another for the transport system but they do not compete with members of other groups. The three main groups are:

1. Neutral amino acids with a free carboxyl group and both an amino group and a hydrogen on the α-carbon atom. The L-isomers are preferentially transported.
2. L-cystine and the basic amino acids, lysine, arginine, and ornithine.
3. Proline and hydroxyproline.

No active transport system has yet been demonstrated for the dicarboxylic amino acids, aspartic and glutamic acid.

Injection of foreign protein directly into the blood produces antibodies which can be detected; oral ingestion of protein does not however result in such antibody formation. Absorption of even traces of protein through the walls of the intestine may give rise to allergic symptoms. The immunologic methods lead to the view that very small quantities of protein (traces) are absorbed as such by some persons and such absorption may be related to sensitivity to special protein foods, such as, egg white. It is known that a protein is antigenic, that is, it is capable of stimulating an immunologic response, only if it is in the form of a relatively large molecule. The digestion of a protein even to the stage of the polypeptide destroys its antigenic property. Absorption of some unhydrolyzed protein by some individuals causes the immunologic response to ingested protein.

Further Reading

B. Harrow and A. Mazur, *Text Book of Biochemistry* (New York: Saunders, 1958).

D.F. Horrobin, *Medical Physiology and Biochemistry* (London: Edward Arnold, 1968).

E.T. Mertz, *Elementary Biochemistry* (Bombay: Vakils, Feffer and Simons, 1967).

G.H. Bell, J.N. Davidson, and D.E. Smith, *Text Book of Physiology and Biochemistry* (London: Livingstone, 1972).

V.G. Dethier, *et al.*, *Topics on the Study of Life: The Biosource Book*, (London: Harper Row, 1971).

H.A. Harper, *Review of Physiological Chemistry* (London: Lange, 1969).

P.B. Weisz, *The Science of Biology* (New York: McGraw-Hill, 1971).

TEN

Metabolism

Introduction

The nutrients eaten as food pass through the alimentary canal to the point where the products of hydrolysis are absorbed and appear in the blood stream. Once in the blood stream, absorbed nutrients are distributed to the cells of the body, where they undergo many remarkable changes. The sum total of these changes are referred to as metabolism. Metabolism includes the energy-requiring synthesis of new complex organic compounds similar to those previously digested as well as the energy-releasing degradation of absorbed nutrients to such simple end-products as carbon dioxide and water. The first aspect of metabolism is known as anabolism and the second as catabolism.

These studies include the transformation of relatively simple compounds, produced during the digestive process, to a variety of intermediate substances until the final end-products are reached. Enzymes bring about these changes, involving the mechanism by which the energy in the chemical bond, created by photosynthetic activity, is transformed so that it may do the kind of work associated with the living cell. This energy may result in heat production, so that the enzymic reactions can occur at high speed; it may be transformed into mechanical energy as happens in a muscle contraction; it may change into electrical energy as shown by the conduction of nerve impulse; or it may transform into light energy, illustrated by firefly or the luminescent micro-organisms of the sea.

The human diet is variable, carbohydrates, however, account for a large proportion of the daily intake. Much of the dietary carbohydrate is converted to fat and consequently metabolized as fat. The process is called *lipogenesis*. The frequency of taking meals and the extent of conversion of carbohydrates to fat may have some influence in producing such disease states as atherosclerosis, obesity, and diabetes mellitus in man.

Synthesis is an important aspect of cellular metabolism by which relatively small molecules join together to form special compounds needed by the cell for special purposes. such as enzymes, hormones and tissue proteins. These synthetic reactions require energy, the catabolic reactions in the cells provide such energy.

Carbohydrate Metabolism

The most important function of carbohydrates is probably to provide energy in a form that the animal or plant can use for other metabolic processes. The cells utilize carbohydrates mainly in the form of glucose. The digestive processes transform the complex carbohydrates into three principal monosaccharides—glucose, fructose, and galactose. Large intake of the disaccharide, sucrose, may result in the production of fructose with considerable quantitative importance. With lactose as the principal carbohydrate of the diet, galactose is of major quantitative significance. Both fructose and galactose are readily converted to glucose by the liver.

Pentose sugars such as xylose, arabinose, and ribose may occur in the diet, but their fate after absorption is obscure. D-ribose and D-2-deoxyribose are synthesized in the body for incorporation into nucleotides.

One gram molecular weight (180 g) of hexose yields 686 k cal (heat required to raise the temperature of 1 kg of water from 15°C to 16°C) of heat when burned to CO_2 and water:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 686 \text{ cal.}$$

The biological form of combustion is called respiration The cell however, needs only a small part of the energy as heat energy. Most of the energy released by respiration is stored in the form of high energy bonds, particularly those found in adenosine triphosphate (ATP). This the cell can use for muscle contraction, electrical energy of nerve impulse, synthesis of new compounds, etc.

The oxidation or burning of carbohydrates to the final end-products, carbon dioxide and water, is a step-wise process, permitting the cell to convert most of the stored energy into chemical bond energy instead of having it all lost as heat.

The metabolism of carbohydrate in the mammalian organism may be subdivided into:

1. *Glycolysis.* This involves the oxidation of glucose or glycogen to pyruvate and lactate by the Embden-Meyerhof pathway.

2. *Glycogenesis.* This pertains to the synthesis of glycogen from glucose.

3. *Glycogenolysis.* This refers to the breakdown of glycogen, glucose

being the main end-product in the liver; and pyruvate and lactate are the main end-products in muscle.

4. *The citric acid cycle.* (Krebs cycle or the tricarboxylic acid cycle.) This involves the final common pathway of oxidation of carbohydrate, fat, and protein through which acetyl-CoA is completely oxidized to CO_2 and water.

5. *The hexose monophosphate shunt (HMS).* This pertains to an alternative pathway to the Embden-Meyerhof pathway and the citric acid cycle for the oxidation of glucose to CO_2 and water.

6. *Gluconeogenesis.* This involves the formation of glucose or glycogen from noncarbohydrate sources. The citric acid cycle and glycolysis are involved in gluconeogenesis, the principal substrate being glucogenic amino-acids, lactate, and glycerol, and propionate in the ruminant.

CONVERSION TO GLYCOGEN

The first step in glucose metabolism is the conversion of absorbed glucose, fructose, and galactose to glycogen in the liver, and blood glucose to glycogen in the muscles. All the three monosaccharides must first combine with phosphate supplied by adenosine triphosphate (ATP) in order to become activated for biological burning. With the transfer of one of the phosphate radicals of ATP to the sugar, some of the energy of the phosphate bond is also transferred:

$$\text{D-Glucose} \xrightarrow[\text{Hexokinase, } Mg^{++}]{\text{ATP} \quad \text{ADP}} \text{D-Glucose-6-phosphate}$$

$$\text{D-Fructose} \xrightarrow[\text{Hexokinase, } Mg^{++}]{\text{ATP} \quad \text{ADP}} \text{D-Fructose-6-phosphate}$$

$$\text{D-Galactose} \xrightarrow[\text{Galactokinase, } Mg^{++}]{\text{ATP} \quad \text{ADP}} \text{D-Galactose-1-phosphate}$$
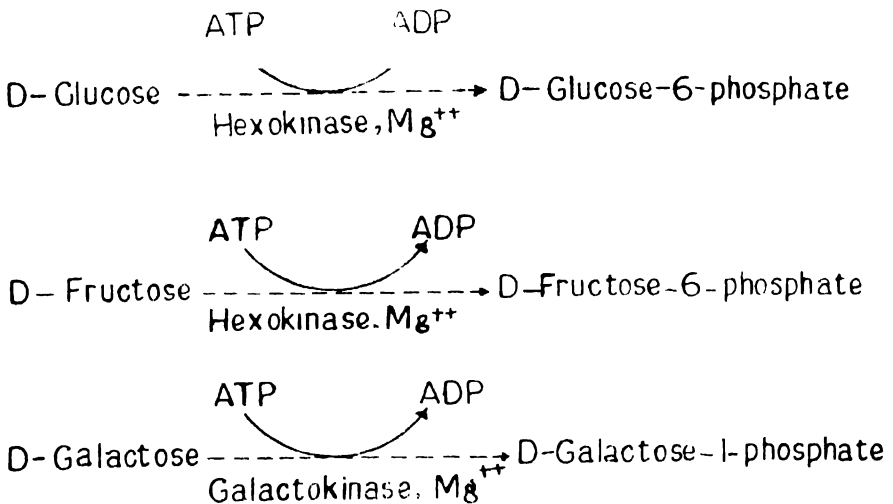
The three phosphated sugars are then converted to D-glucose-1-phosphate:

$$\text{-D-glulose-6-phosphate} \xrightarrow{\text{phosphoglucomutase}} \text{D-glucose-1-phosphate}$$

$$\text{D-fructose-6-phosphte} \xrightarrow{\text{phosphohexoseisomerase}} \text{D-glucose-6-phosphate---}$$

$$\text{D-galactose-1-phosphate} \xrightarrow[\text{(Coenzyme: UDPG}]{\text{uridyltransferase}} \begin{array}{c}\text{D-glucose-1-phosphate}\\ \text{+ UDP-galactose}\end{array}$$

$$\text{UDP-galactose} \xrightarrow{\text{galactowaldenase}} \text{UDPG (Uridine-diphosphate-}$$
$$\text{glucose}$$
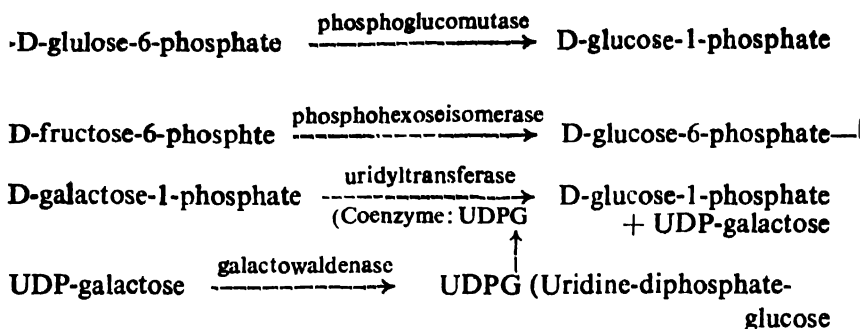
Fructose-6-phosphate is first isomerized to glucose-6-phosphate before the phosphate shift from carbon 6 to carbon 1 takes place. Galactose is isomerized to glucose after conversion to UDP-galactose, in two steps.

The D-glucose-1-phosphate (Cori ester) formed, serves as the building unit for liver or muscle glycogen.

$$\text{UDPG} + (\text{D-glucose-1-phosphate})_n \xrightarrow[\text{branching enzyme}]{\text{glycogen synthetase}} (\text{glucose})_{n+1}$$
$$+ (\text{PO}_4{}^{---})_n + \text{UDP[amylo (1,4} \rightarrow \text{1,6) transglucosidase]}$$
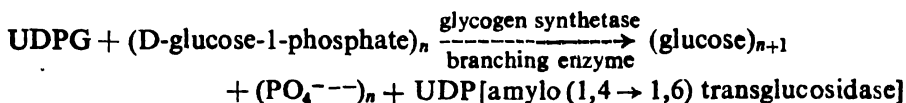
In the synthesis, glycogen synthetase, with UDPG as coenzyme, adds glucose to the nonreducing end of the chain, building straight chains. When the straight chains reach a length of approximately 8 glucose units, they are acted on by the branching enzyme, converting some α-1,4 linkages to α-1,6 linkages. This results in branching on a glucose unit which still contains a carbon 4 linkage.

CONVERSION OF GLYCOGEN TO GLUCOSE

The second step in the metabolism of carbohydrates involves the conversion of glycogen to glucose:

Liver glycogen   Muscle glycogen

$$\text{PO}_4{}^{---} \Big\downarrow \begin{array}{l}\text{liver}\\ \text{phosphorylase}\end{array} \qquad \text{PO}_4{}^{---} \Big\downarrow \begin{array}{l}\text{muscle}\\ \text{phosphorylase}\end{array}$$

Glucose-1-phosphate   glucose-1-phosphate

$$\Big\downarrow \text{phosphoglucomutase} \qquad\qquad \Big\downarrow \text{phosphoglucomutase}$$

glucose-6-phosphate   glucose-6-phosphate.
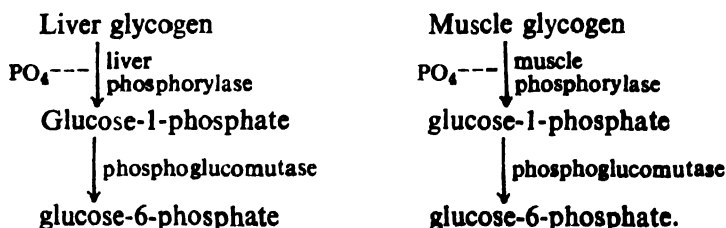
Synthesis of glycogen involves synthetase, branching enzyme, and phosphoglucomutase. Phosphorylase-a is needed for the degradation of glycogen. The relative abundance of glucose in the blood, and the relative influence of the hormones insulin, epinephrine, and glucagon determine whether glucose will be converted to glycogen or glycogen to glucose.

Insulin

The hormone stimulates the conversion of glucose to liver and muscle glycogen. The exact mechanism is not known. It may be that the insulin speeds up the transfer of glucose across the cell membrane to the site of glycogen synthesis. Another possibility is that the insulin accelerates the hexokinase reaction. The hexokinase acceleration theory appears to be more plausible as evident from the act that the pituitary secretes a hormone (diabetogenic) inhibiting the action of hexokinase. Hussay found that the removal of the pituitary in a diabetic animal eliminates the need for insulin injection.

Epinephrine

This stimulates the conversion of glycogen to glucose, probably by accelerating the phosphorylase enzyme activity. Phosphorylase has been found in liver and muscle in two forms, *a* and *b*, *a*-form being active and *b*-form inactive. Adenylic acid can activate the *b*-form. It is probable that epinephrine stimulates the conversion of phosphorylase *b* to phosphoylase *a*.

Glucagon

Conversion of glycogen to glucose is stimulated by glucagon. It does not have significant effect on muscle glycogen. It appears to accelerate liver phosphorylase activity by conversion of the inactive *b*-form to the active *a*-form.

In addition to insulin, found in the beta cells of the pancreas, another crystalline protein hormone, glucogon, which is apparently elaborated by the alpha cells, has been isolated from the pancreas. Glucagon has an effect opposite to that of insulin; it results in a h_ erglycemia, a rise in blood glucose.

GLUCOSE-6-PHOSPHATE TO PYRUVIC ACID AND LACTIC ACID

Little energy changes are involved in the first two steps of carbohydrate metabolism wherein monosaccharides are converted into glycogen and then to glucose. Approximately 4 kcal of energy per mole (gram molecular weight) of glucose are required for the synthesis or splitting of the glycosidic bonds of glycogen, representing less than 1 per cent of the energy released by the complete combustion of one mol of glucose to carbon dioxide and water.

The third step, which may be called anaerobic glycolysis or fermentation, gives rise to about 57 kcal of energy per mole of glucose, about 8 per cent of the total stored energy of glucose molecule when glucose is converted to lactic acid. About one-fourth of this energy is recovered as chemical bond energy as two ATP molecules are synthesized from one molecule of ADP per mole of glucose:

Glucose-6-phosphate

Phosphohexose isomerase
(animal or yeast cells)

Fructose-6-phosphate

ATP
ADP $\rightleftharpoons$ phosphohexokinase

Fructose-1,6-diphosphate

aldolase
(zymohexase yeast)

Phosphodihydroxy
acetone

Phosphotriose isomerase

3 phosphoglyceraldehyde

Triose phosphate-
dehydrogenase

PO_4^{---}, 2 NAD$^+$ 2 NADH+H$^+$

1,3-diphosphoglyceric acid (2 mols)

ADP
ATP $\rightleftharpoons$ phosphokinase

3-phosphoglyceric acid (2 mols)

phosphoglyceromutase

2-phosphoglyceric acid (2 mols)

enolase

phosphoenol-pyruvic acid (2 mols)

ADP
ATP $\rightleftharpoons$ phosphokinase

enol-pyruvic acid (2 mols)

rearrange

pyruvic acid (2 mols)

TPP, Hg^{++} yeast cells
yeast carboxylase

Acetaldehyde + CO$_2$
H$^+$, NADH
NAD $\rightleftharpoons$ alcoholdehydro
genase

ethylalcohol (2 mols)

H$^+$, 2 NADH
2 NAD $\rightleftharpoons$ muscle or liver
lactic dehydrogenase

Lactic acid (2 mols)

The Embden-Meyerhof
pathway of glycolysis

Glucose-6-phosphate
(α-form)

$$
\begin{array}{c}
\text{H} \quad \text{OH} \\
| \quad | \\
\text{C} \\
/ \quad \backslash \\
\text{H} - \text{C} - \text{OH} \\
\text{HO} - \text{C} - \text{H} \\
\text{H} - \text{C} - \text{OH} \\
\text{H} - \text{C} \\
\text{H} - \text{C} - \text{O} - \text{P} = \text{O} \\
\quad \quad \text{OH} \\
\quad \quad \text{OH}
\end{array}
$$

Fructose-6-phosphate
(α-form)

Fructose-1,6 diphosphate
(α-form)

Phosphodihydroxy-acetone

$$
\begin{array}{c}
\text{H} - \text{C} - \text{H} \\
\text{H} - \text{C} - \text{O} - \text{P} = \text{O} \\
\quad \quad \text{OH} \\
\quad \quad \text{OH} \\
\text{C} = \text{O} \\
\text{H} - \text{C} - \text{OH} \\
\text{H}
\end{array}
$$

3-phosphoglyceraldehyde

1,3-diphosphoglyceric acid

Nine enzymatically catalysed steps are involved in the conversion of glucose-6-phosphate to pyruvic acid. Formation of lactic acid from pyruvic acid needs another step. The conversion of glucose-6-phosphate to pyruvic acid follows the same pathway in the muscle and liver of animals as in the yeast microorganism. From the steps it is evident that glucose to alcohol formation brings about a net gain of two molecules of ATP per mol of glucose fermented. The yeast cells derive their energy from the gain in high energy bonds, fermenting relatively large amounts of glucose.

3-phosphoglyceric acid   2-phosphoglyceric acid   2 phosphoenol-pyruvic acid

Enol pyruvic acid   Pyruvic acid   Lactic acid
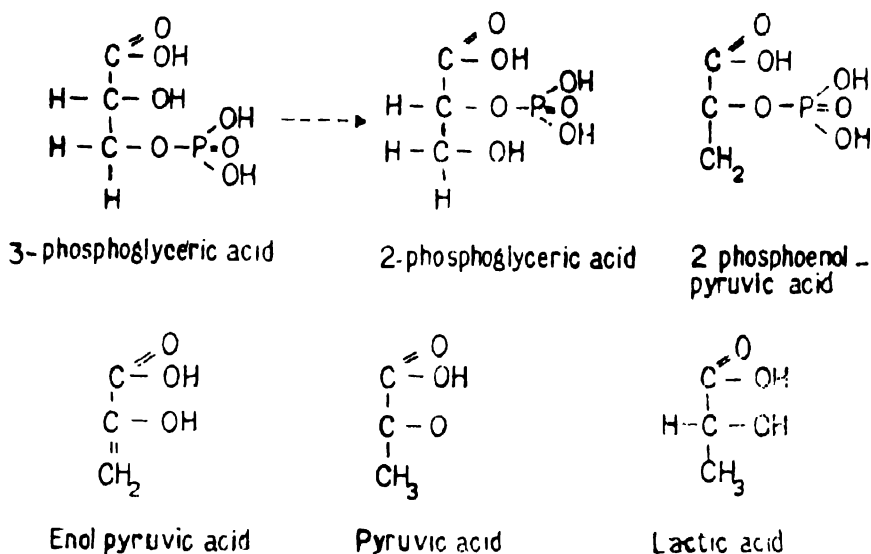
ALTERNATE PATHWAYS FOR GLUCOSE OXIDATION: THE HEXOSE MONOPHOSPHATE SHUNT

The reactions of glycolysis are referred to as the Embden-Meyerhof Pathway, as indicated above. Studies on plants, bacteria, and some mammalian tissues have indicated an alternate pathway of glucose oxidation, the direct oxidative pathway, or the Warburg-Lipmann-Dickens Pathway.

Under the Embden-Meyerhof scheme, molecular oxygen is not required upto lactic acid and therefore the scheme is capable of acting anaerobically. In the alternate pathway, molecular oxygen is required in the early part of the reaction, and TPN is used.

Modified hexose monophosphate shunt is shown below:

Glucose- 6 phosphate

$\frac{1}{2}O_2$ | TPN

(1) | TPNH

6-Phosphogluconolactone   (8)

(2) |

6-Phosphogluconic acid

$\frac{1}{2}O_2$ | TPN   Fructose-6 phosphate + Erythrose-4-phosphate

(3) | TPNH

(3 Keto-6-phosphogluconic acid

(4) | $-CO_2$ (7)

Ribulose-5-phosphate | (6) Sedoheptulose -   + 3 phospho -
                                  7 phosphate   glyceraldehyde
(5) |

Ribose-5-phosphate
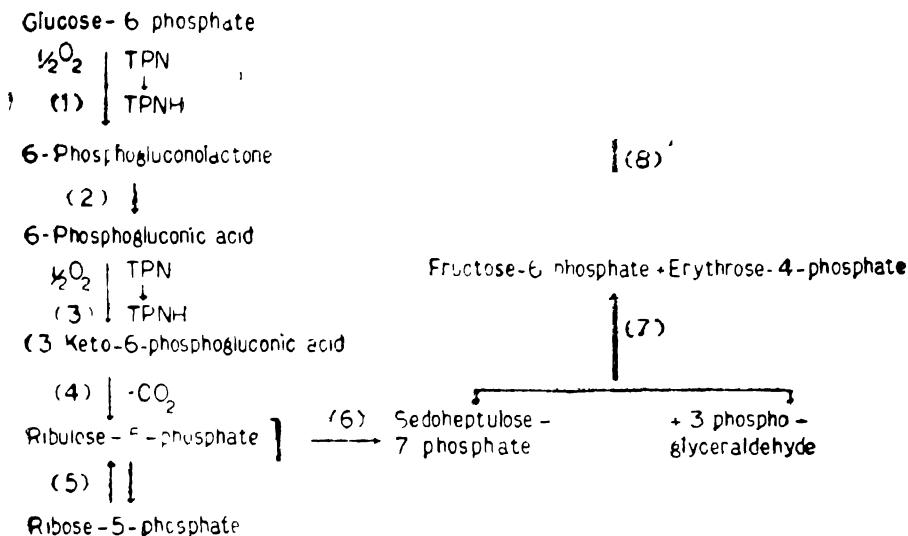
Enzymes are involved in the hexosemonophosphate shunt are:

Reaction	Enzyme
(1)	Glucose-6-phosphate dehydrogenase
(2)	6-phosphogluconolactonase
(3)	6-phosphogluconic dehydrogenase
(4)	Spontaneous
(5)	Phosphopentose isomerase
(6)	Transketolase
(7)	Transaldolase
(8)	Phosphohexose isomerase

The reactions are shown by chemical formulas.

Aerobic Glycolysis

Glycolysis leading to the formation of lactic acid, is restricted to anaerobic conditions. The formation of lactic acid is inhibited by the presence of oxygen. This inhibition of glycolysis by oxygen is known as the Pasteur effect.

A theory has been proposed by Lynen and Johnson to explain the Pasteur effect. According to them much more esterification of inorganic phosphate takes place during aerobic cellular metabolism than during anaerobic glycolysis. The concentration of inorganic phosphate in the cell is lowered considerably during aerobic conditions as compared to the anaerobic cell. The aerobic conditions thus favour glycogen synthesis tending to depress glycolysis, the formation of lactic acid.

PYRUVIC AND LACTIC ACIDS OXIDATION TO CO_2 AND WATER

Oxidation (aerobic glycolysis) of pyruvic acid and lactic acid to carbon

dioxide and water results in the release of the greatest proportion of the stored energy of the glucose molecule about 90 per cent of the total. Pyruvate or lactate is burned to carbon dioxide and water in a cycle of enzymatically catalysed steps, about equal in number to those involved in anaerobic glycolysis. The cycle is called the Krebs cycle or the citric acid cycle of the tricarboxylic acid cycle· The steps are indicated in Fig 10.1.

In the Krebs cycle, pyruvate is converted to acetyl CoA, and fed into the cycle as such. In the liver both pyruvate and lactates are carboxylated to form oxaloacetic acid. The chemical formulas of the compounds involved in Krebs cycle are as below:

COOH
|
C = O
|
CH₃
Pyruvic acid

COOH
|
C = O
|
CH₂
|
COOH
Oxaloacetic
acid

→

COOH
|
CH₂
|
HO—C—COOH
|
CH₂
|
COOH
Citric acid

→

COOH
|
CH
‖
C—COOH
|
CH₂
|
COOH
Cis-aconitic acid

→

COOH
|
H—C—OH
|
H—C—COOH
|
CH₂
|
COOH
Isocitric acid

COOH
|
C=O
|
H—C—COOH
|
CH₂
|
COOH
Oxalosuccinic acid

COOH
|
C=O
|
CH₂
|
CH₂
|
COOH
α-Ketoglutaric acid

COOH
|
CH₂
|
CH₂
|
COOH
Succinic acid

COOH
|
HC
‖
CH
|
COOH
Fumaric acid

COOH
|
C—H₂
|
H—C=OH
|
COOH
Malic acid
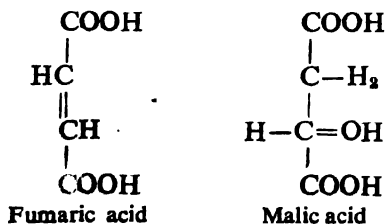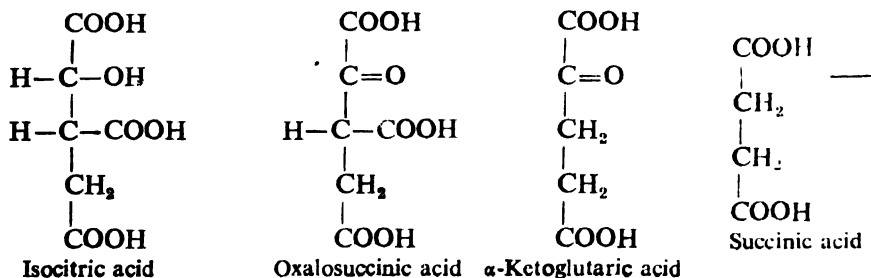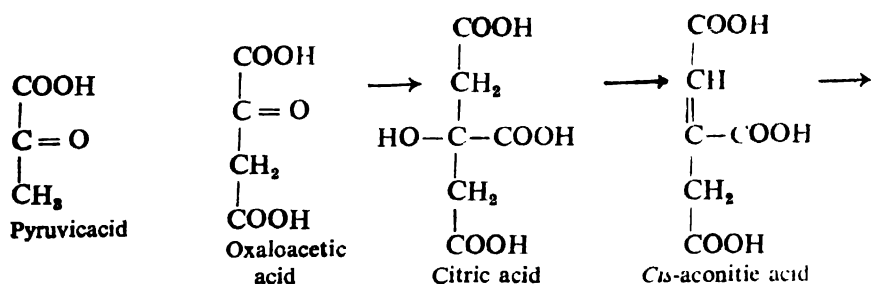
Conversion of pyruvic acid to acetyl coenzyme A is a complicated reaction. The pathway involves many factors:

Conversion of pyruvic acid to acetyl coenzyme.
reaction. The pathway involves many factors:

Pyruvate — Liver muscle — Lactate

NAD^+
$H + NADH$

CO_2

CoA, pyruvic acid
dehydrogenase,
TPP, lipoic acid

Acetyl CoA

$(+CO_2)$

Oxaloacetic acid

β-Carboxylase (liver only)
thiamine pyrophosphate

$(+H_2O)$
(1)

Citric acid

$(-H_2O)$
(2)

Aconitase

Cisaconitic acid

$(+H_2O)$
(3)

Aconitase

Isocitric acid

$NADP^+$
$H^+ + NADPH^+$
.(4)

Isocitric dehydrogenase

Oxalosuccinic acid

Carboxylase

(5)

α−Ketoglutaric acid

Malic dehydrogenase — Malic acid

NAD^+ $NADH$
$+ H^+$

Fumarase
$(+H_2O)$
(8)

Fumaric acid

$FADH_2$
FAD
(7)

Succinic
dehydrogenase

Succinic acid

$H^+ + NADH$
NAD^+
(6)

CO_2
$(-H_2O)$

Fig. 10.1 The Kreb's cycle.

$$
\begin{array}{c}
\text{COOH} \\
| \\
\text{C=O} \\
| \\
\text{CH}_3
\end{array}
\quad + \text{TPP} +
\begin{array}{c}
\text{S} \\
| \\
\text{S} \\
| \\
\text{COOH}
\end{array}
\longrightarrow
\begin{array}{c}
\text{CH}_3\text{CS} \\
\; \| \\
\text{O} \\
| \\
\text{COOH}
\end{array}
\begin{array}{c}
\text{HS} \\
\\
\end{array}
+ CO_2 + \text{TPP}
$$

Pyruvic acid   Lipoic acid   Acetyl lipoic acid   Thiamine pyrophosphate

$$
\begin{array}{c}
\text{CH}_3\text{C} - \text{S} \\
\; \| \\
\text{O} \\
\end{array}
\begin{array}{c}
\text{HS} \\
| \\
\text{COOH}
\end{array}
+ \text{CoASH}
$$

Acetyl lipoic acid   Coenzyme

$$
\begin{array}{c}
\text{O} \\
\| \\
\text{CH}_3\text{C} - \text{SCoA}
\end{array}
+
\begin{array}{c}
\text{HS} \\
\text{HS} \\
| \\
\text{COOH}
\end{array}
$$

Acetyl CoA   Reduced lipoic acid

$$
\begin{array}{c}
\text{HS} \\
\text{HS} \\
| \\
\text{COOH}
\end{array}
+ \text{NAD}^+ \longrightarrow
\begin{array}{c}
\text{S} \\
\text{S} \\
| \\
\text{COOH}
\end{array}
+ \text{NADH} + \text{H}^+
$$

Reduced lipoic acid   Oxidized lipoic acid

Pyruvic acid reacts with lipoic acid (an eight carbon straight chain fatty acid with sulphur atoms at positions 6 and 8) in presence of pyruvic acid dehydrogenase with thiamine pyrophosphate as coenzyme. Acetyl lipoic acid is formed and CO_2 is released. Acetyl CoA is formed by a transfer of the acetyl group to CoASH with the production of reduced lipoic acid. The latter is then oxidized to the ring form by NAD^+.

Acetyl CoA is a high energy compound capable of acting either as a biological acetylating agent or in condensation reactions. The methyl group of acetyl CoA is activated condensing with the ketogroup of oxalacetic acid in presence of condensing enzyme The reaction (1) is reversible, but the equilibrium mixture contains a high percentage of citric acid.

An iron (Fe^{++})-containing enzyme, aconitase, catalyzes the conversion of citric acid to cis-aconitic as well as to iso-citric acids [reactions (2) and (3)]. The respective amounts of the three acids at equilibrium are 90, 4 and 6 per cent. In respiring tissues the reaction proceeds in the direction of isocitric acid as it is removed in the next reaction of the cycle. Aconitase is believed t attach to three of the four groups of citric acid, making its centre carbon a symmetric during dehydration reaction giving cis-aconitic acid.

The formation of oxalosuccinic acid involves removal of hydrogen by the coenzyme ʋADP⁺ in presence of the apoenzyme, isocitric dehydrogenase, the reaction (4) being reversible.

Oxalosuccinic acid loses a molecule of CO_2 in presence of the enzyme, cocarboxylase. to form α-ketoglutaric acid. The reaction (5) is reversible. α-ketoglutaric acid is converted to succinic acid through a two-stage reaction (6) involving the formation of succinyl C . ʻ. as in intermediate which is analogous to the oxidative decarboxylation of pyruvic acid.

$$
\begin{array}{l}
CH_2 \cdot COOH \\
| \\
CH_2 \quad CoASH \cdot TPP, Mg^{++} \text{ lipoic acid} \\
| \qquad\qquad \overline{\qquad\qquad\qquad\qquad} \\
O = C \cdot COOH \qquad NAD^+ \quad NADH + H^+
\end{array}
\longrightarrow
\begin{array}{l}
CH_2 - COOH \\
| \qquad O \\
CH_2 \cdot C \nearrow SCoA + CO_2
\end{array}
$$

α - Ketoglutaric acid
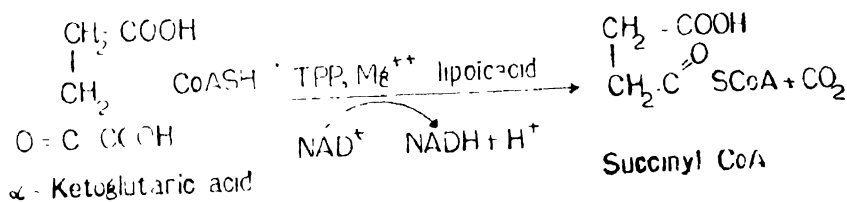
Succinyl CoA

Thiamine pyrophosphate (TPP) condenses with α-ketoglutaric acid releasing CO_2 and forming α-hydroxy-γ-carboxy propyl ʻhiamine pyrophosphate. The succinyl group is transferred from the thiamine to enzyme-bound lipoic acid. Succinyl lipoic acid reacts with CoASH to give succinyl CoA and reduced lipoic acid, which is oxidized by NAD^+ to the cyclic form. The lipoic acid is probably bound to the enzyme by amide linkage with ϵ-amino group of lysine in this reaction as well as that with pyruvic acid.

The thioester energy of Succinyl CoA may be utilized to initiate fatty acid oxidation, for acylation reactions, for porphyrin synthesis by condensation with glycine. But most of the succinyl CoA formed in the citric acid

cycle is utilized for the synthesis of additional ATP. Succinyl CoA reacts with guanosine diphosphate and inorganic phosphate to form succinic acid, GTP, and CoASH. The GTP reacts with ADP to produce GDP and ATP. The succinic acid thiokinase and nucleoside diphospho-kinase catalyze these two reactions respectively. The formation of Succinpl CoA is not reversible preventing the reaction in the reverse direction.

Succinic acid dehydrogenase catalyses the dehydrogenation of succinic acid.-a reaction in the citric acid cycle, in which pyridine nucleotides, NAD^+ and NADP, are not involved. This enzyme contains four atoms of iron and one mole of flavin per mole of protein, with a molecular weight of 200,000. The enzyme produces only the trans form of fumaric acid and the cis form, maleic acid. The reaction (7) is reversible. Malonic acid, $HOOC-CH_2-COOH$, is a specific competitive inhibitor of the oxidation of succinic acid. Malonate can therefore be used to interrupt the citric acid cycle with resultant accumulation of succinate.

The hydration of fumaric acid to malic acid is catalysed by the enzyme fumarase. The reaction (8) is freely reversible, fumarate being present in most abundance of all the members of the citric acid cycle in mammalian tissues.

Malic acid is oxidized to oxaloacetic acid by NAD^+ in presence of malic dehydrogenase and the cycle is completed, the generated oxaloacetic acid is available for condensation with another mole of acetyl CoA. The reaction (9) $\underset{\sim}{\approx}$ reversible.

ENERGETICS OF CARBOHYDRATE OXIDATION

About 686,000 calories are liberated as heat when 1 mol of glucose is combusted in a calorimeter to CO_2 and water. Oxidation of carbohydrate in the tissues does not permit some of this energy to be lost immediately as heat, instead it is captured in high energy phosphate bonds. With the oxidation of one molecule of glucose to CO_2 and water, at least 38 high-energy phosphate bonds are generated. It can be assumed that each high-energy bond is equivalent to 7,600 calories and thus the total energy captured in 38 ATP generated per mole of glucose oxidation is 288,800 calories or approximately 42 per cent of the energy of combustion. Most of the ATP is formed from oxidative phosphorylation resulting from the reoxidation of reduced coenzymes by the respiratory chain. The remainder is formed by phosphorylation at the substrate level. The generation of the new high-energy bonds takes place in the reactions indicated in Table 10.1.

TABLE 10.1   CATABOLISM OF GLUCOSE WITH THE GENERATION OF HIGH-ENERGY BONDS

Pathway	Reaction catalysed by	Method of $\sim P$ production	No. of $\sim P$ formed per mol of glucose
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	Phosphoglycerate kinase	Oxidation at substrate level	2
	Pyruvate kinase	Oxidation at substrate level	2
			10
	ATP consumption by reactions catalyzed by hexokinase and phosphofructokinase		-2
			Net 8
Citric acid cycle	Pyruvate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	Isocitrate dehydrogenase	Respiratory chain oxidation of 2NADH	6
	α-Ketoglutaratedehydrogenase	Respiratory chain oxidation of 2NADH	6
	Succinate thiokinase	Oxidation at substrate level	2
	Succinate dehydrogenase	Respiratory chain oxidation of $2FADH_2$	4
	Malate dehydrogenase	Respiratory chain oxidation of 2NADH	6
			Net 30
Total per mol of glucose under aerobic conditions			38
Total per mol of glucose under anaerobic conditions			2

The metabolism of glucose and glycogen varies from tissue to tissue but the major metabolic patterns are as below:

1. Complete oxidation to CO_2 and water by glycolytic pathway and the citric acid cycle yields approximately 38 molecules of ATP per molecule of glucose. This is the main pathway used in brain and peripheral nerve.

2. Anaerobic conversion to lactate in tissues lacking either mitochondria or an adequate oxygen supply, generates only 2 molecules of ATP (3 if the glucose is in the form of glycogen). The lactate however can be oxidized to provide energy in other tissues.

3. Complete oxidation to CO_2 and water by the pentose phosphate pathway is sufficient to reduce 12 molecules of NADP per molecule of glucose. This is of importance in adipose tissue where large quantities of $NADPH_2$ are required for the synthesis of fatty acids.

The interconversion of glucose, glycogen, and galactose is indicated in Fig. 10.2.

Children with a congenital lack of phosphogalactose uridly transferase cannot metabolize galactose resulting in the hereditary disease galactosaemia in which galactose and galactose-1 phosphate accumulate in the blood and are excreted in the urine.

The carbohydrate provided by digestion in the gut can be stored until it is needed. A limited amount is stored in the form of glycogen either in the liver or in the muscles. Liver glycogen can be broken down again to free glucose which can be made available to all the tissues by way of the hlood stream. Muscle glycogen is however available only to the cell in which it is located. Alternatively, circulating glucose can be taken up by the adipose tissue and converted to triglycerides. The fuel reserves in the adipose tissues are very much larger than those stored as glycogen in liver and muscle. All cells and tissues require energy, carbohydrate in some degree being utilized by all or almost all. A few organs like brain, skeletal muscle, heart muscle, liver, kidney, and the red and white blood cells are of outstanding importance in this regard. Carbohydrate is used by each of them in a different and characteristric manner. The nervous tissue constitutes the simplest pattern. The brain has a constant requirement for energy about 25 per cent of the energy requirement of a normal resting adult. This is met almost entirely by the conversion of carbohydrate to CO_2 and water of approximately 120 g of glucose per day. This requirement is extraordinarily constant unaffected by intellectual exertion or by sleep. It is maintained even when the blood glucose level is moderately depressed. Interruption of the supply of oxygen or of glucose on the other hand even for a brief period results in irreversible brain damage. The glycogen content in brain is too small to meet the requirement. The energy requirements of the erythrocytes and leucocytcs with a total mass (2.5 kg) far exceeding that of the brain, though constant like those of brain, are quite small. They are met by the conversion to lactate of about 36 g of glucose per day, erythrocytes having no mitochondria. The energy requirement of skeletal muscle, unlike nervous tissue and erythrocytes, is extraordinarily variable being metabolically fairly incrt in the resting subject. The modest energy requirement is probably met largely from the oxidation of fat rather than carbohydrate.

Running or other vigrous exercise on the other hand is accompanied by a greatly increased blood supply providing the numerous muscle mitochondria with a correspondingly increased oxygen supply.

Glucose (extra cellular)

- - - - - Cellmembrane

Glucose (intracellular)

ATP
Glucokinase
ADP

$H_2O + CO_2$

Pentosephosphate pathway

Glucose-6-phosphatase

Glucose-6-Phosphate

Phospho glucomutase

Glycogen Phosphorylase

Fructose-6-phosphate

ATP
ADP

Fructose-1,6-diphosphate

Glycolysis

Pyruvate ⇌ Lactate

Citric acid cycle

$CO_2 + H_2O$

Glucose-1-phosphate

UDP Gal
UDP Glu

Phosphide galactose uridlytransferase

Glucose-1-phosphate

ADP
ATP

Galacto kinase

Galactose

Fig. 10.2

The enormously increased energy output is met not by accelerating the existing process of fatty acid oxidation but by the oxidation of carbohydrate—some derived from blood and the major portion from the muscles own store of glycogen. With the reserve used up, blood sugar level declines sharply accompanied by fatigue. Administration of a quite small amount of glucose relieves the fatigue markedly raising the blood sugar. The muscles continue to depend on their supply of fatty acids. Anaerobic glycolysis can be an important source of energy especially in brief periods of very vigorous exertion with inadequate oxygen supply to meet the demand made on the muscles. The production of ATP by glycolysis is accompanied by the liberation of large amounts of lactic acid. This is indicated in the Cori cycle:

                        Glucose in blood
glycogeno-   ↗   ↘   glycogenesis
lysis   ╱   ↘
          ╱   ↘
Glycogen in liver   Glycogen in muscle
    ↖   ╱
gluco-   ↖   ╱
neogenesis ↖ Lactic acid ↙ glycolysis
              in blood
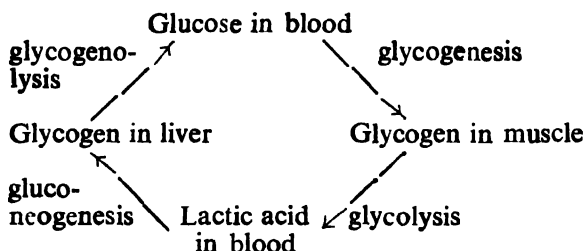
Liver is important in carbohydrate metabolism. In stores the carbohydrate metabolism. It stores the carbohydrate absorbed in the intestine, in the form of glycogen, about 90 g or half a day's supply of carbodydrate for the entire organism in the liver of a well nourished man. The glycogen can be broken down at need and liberated as free glucose in the blood for use by all the tissues, particularly the brain. This stored carbohydrate in the form of liver glycogen prevents hypoglycaemia under normal conditions. Von Gierke's disease, a rare genetic deficiency, is caused by the lack of the enzyme glucose-6-phosphatase so that although glycogen is formed in the liver, it cannot be converted to glucose for use by other tissues. Liver glycogen can be regarded as a fuel tank for the brain.

Another important role of the liver is in gluconeogenesis, which meets the needs of the body for glucose when carbohydrate is not available in sufficient amounts from the diet. The formed elements of the blood convert approximately 36 g glucose a day to lactate and the muscles can produce 50 or even 100 g lactate in a few minutes in vigorous exertion. Other tissues such as the heart, and perhaps by resting skeletal muscles take up some of this lactate to be oxidized to CO_2 and water. About half is converted to glucose or glycogen in the liver by reversal of the glycolytic pathway-the Cori cycle. The rate of this process is determined on the concentration of lactate in blood.

A continual supply of glucose is necessary as a source of energy especially for the nervous system and the erythrocytes. Glucose is also required in adipose tissue as a source of glyceride-glycerol and it probably

plays a role in maintaining the level of intermediates of the citric acid cycle in many tissues. It is the precursor of milk sugar (lactose) in the mammary gland it is taken up actively by the foetus. The enzymatic pathways have been developed in certain specialized tissues for the conversion of non-carbohydrates to glucose. The liver is capable of forming glucose or glycogen from amino acids: Pathways of gluconeogenesis indicating the formation of glucose from lactate and glucogenic aminoacids are shown below. Oxaloacetate is a key intermediate in both pathway (see Fig. 10.3).

In addition, these gluconeogenic mechanisms are utilized to clear the products of the metabolism of other tissues from the blood, like lactate, produced by muscle and erythrocytes, and glycerol, continuously produced by adipose tissues.

The liver and the kidney are the principal organs responsible for gluconeogenesis. Gluconeogenesis is essentially the reversal of glucolysis. The glycolytic activity of the liver and kidney is therefore low when active gluconeogenesis take place.

The glucose formed as a result of gluconeogenesis is important for brain in brief periods of fasting. The liver does not use carbohydrate as an energy source extensively. It appears to subsist mainly by the oxidation of amino acids in a normal well-fed individual and in starvation, when amino acids are required for glucose formation, the liver can maintain itself by the oxidation of fatty acids.

The metabolic pathways involved in gluconeogenesis are modifications and adaptations of the Embden-Meyerhof pathways and the citric acid cycle. They are concerned with the conversion of glucogenic amino acids, lactate, glycerol, and in ruminants, propionate, to glucose or glycogen.

The enzymes, pyruvate carboxylase, occurs in the mitochondria and is responsible for the conversion of pyruvate to oxaloacetate in presence of ATP, biotin, and CO_3. In the extramitochondrial part of the cell a second enzyme, phosphoenol pyruvate carboxykinase is found, capable of catalyzing the conversion of oxaloacetate to phosphoenol pyruvate. High energy phosphate in the form of GTP is required in this reaction and CO_2 is liberated. These two enzymes, convert lactate to phosphoenol-pyruvate. Oxaloacetate, however, does not diffuse readily from mitochondria, which becomes possible by conversion of oxaloacetate into compounds which can diffuse from the mitochondria followed by their reconversion to oxaloacetate in the extramitochondrial portion of the cell. These compounds are malate, asparatate, α-ketoglutarate, glutamate, and citrate. The citric acid cycle reactions and transmination reactions are involved in their formation from oxaloacetate within mitochondria and in their conversion back to oxaloacetate in the extra-mitochondrial part of the cell.

The enzyme, fructose-1, 6-diphosphatase, catalyses the conversion of fructose-1,6-diphosphate to fructose-6-phosphate, necessary for the reversal of glycolysis. This enzyme determines whether or not a tissue is

Amino acids

Lactate

Pyruvate

Phosphoenol
pyruvate

Triose phosphate

Fructose-1,6-diphosphate

AMP
activates inhibits

Fructose-6-phosphate

Glucose-6-phosphate

Glucose

ADP
ATP

ADP
ATP

P_i

P_i

Oxaloacetate

CO_2
CO_2

Alanine, serine

Aspartate

Malate

Fumarate

Succinate

α-Oxogluarate

Valine
isoleucine
threonine
glutamate
histidine
arginine,
preline

Citrate

Tricarboxylic
acid cycle

$CO_2 + H_2O$

Fig. 10.3

capable of resynthesizing glycogen from pyruvate and triosephosphates.

The enzyme glucose-6-phosphatase present in liver, intestine and kidney catalyses the conversion of glucose-6-phosphate to glucose. The enzyme allows these tissues to provide glucose to the blood. The enzyme is microsomal, possessing pyrophosphatase activity. Muscle and adipose tissue do not contain this enzyme.

The enzyme phosphorylase catalyses the breakdown of glycogen to glucose-1-phosphate. The synthesis of glycogen follows a different pathway through the formation of uridine diphosphoglucose and the activity of glycogen synthetase.

The breakdown of triglycerides in the adipose tissue provide the fatty acids needed by muscle, liver and kidney. An equivalent of 16 g carbohydrate per day is provided by the process in the form of glycerol. The slow breakdown of muscle protein gives amino acids, most of which are convertible to glucose.

The brain and nervous tissue glucose under prolonged starvation adapt their metabolic pathways to use increasing amounts of fatty acid metabolitis thus sparing the body's carbohydrate resources.

Gluconeogenesis and lipogenesis as they occur in the liver cell, are indicated by the following pathways (Fig. 10.4).

Gucogenic amino acids on transamination or deamination, form either pyruvate or members of the citric acid cycle. The pathways indicate the conversion of both glucogenic amino acids and lactate to glucose or glycogen.

Propionate is a major source of glucose in ruminants. It enters the main gluconeogenic pathway via the citric acid cycle after conversion to succinyl-CoA. Propionate, as with other fatty acids, is first activated with ATP and CoA by an appropriate thiokinase to form Propionyl-CoA. The product undergoes a CO_2 fixation reaction to form D-methylmalonyl-CoA, propionyl CoA carboxylase being the enzyme which needs biotin as a coenzyme. Methyl-malonyl-CoA racemase converts D-Methyl-malonyl-CoA to its sterioisomer, L-methylmalonyl-CoA. The final isomerization to succinyl-CoA is brought about by the enzyme methyl-malonyl-CoA isomerase which needs Vitamin B_{12} as a coenzyme.

Propionate although having its main pathway of metabolism to succinate, may also participate as the priming molecule for the synthesis of fatty acids with odd number of carbon atoms, in adipose tissue and mammary gland. C-15 and C-17 fatty acids are found particularly in the lipids of ruminants. The metabolism of propionic acid is indicated by the following pathway (Fig. 10.5).

The enzyme, glycerokinase, found among other tissues, in liver and kidney, catalyzes the conversion of glycerol to α-glycerophosphate in presence of ATP. Another enzyme, α-glycerophosphate dehydrogenase, catalyses the oxidation of α-glycerophosphate to dihydroxyacetone phosphate in presence of NAD^+ to bring the reaction to the triosephosphate stages of the Embden-Meyerhof pathway. These two enzymes, some of

the enzymes of the E-M pathway, and the specific enzymes of the gluco-neogenic pathway—fructose-1,6-diphosphatase and glucose-6-phosphatase, in the liver and kidney—are thus able to convert glycerol to blood glucose.

Isocitrate dehydrogenase present in the extramitochondrial part of the cell requires NADP for activation. Malic enzyme also present in the extramitochondrial part, brings about the oxidative decarboxylation of malate to pyruvate with the formation of NADPH. These two enzymes augment the extramitochondrial production of NADPH by the hexose monophosphate shunt. They appear to be concerned more with lipogenesis than with gluconeogenesis.

Liver glycogen constitutes the stored carbohydrate of the body and is being regenerated constantly as a result of reactions involving the absorbed monosaccharides and blood glucose. The regulation of glycogen synthesis and breakdown is affected by several hormones, apparently by controlling the activity of enzymes like phosphorylase and hexokinase, or by the regulation of glucose transport. The relationship of muscular contraction to liver glycogen and blood glucose is indicated as below:

Diet carbohydrate
↓
Liver glycogen   →   blood lactic acid
↓ ↑   ↑
Blood glucose   →   Muscle glycogen ⇄ Lactic acid
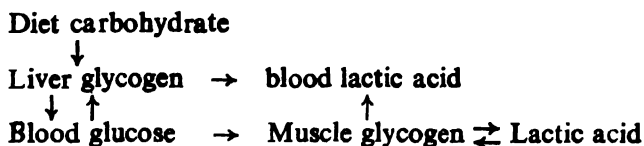
Most carbohydrates in the diet form glucose or fructose on digestion. These are absorbed into the portal vein. Fructose is readily converted into glucose in the liver. Glucogenic compounds on gluconeogenesis are converted into glucose. These compounds may be of two categories: one involved in direct conversion without significant recycling, such as amino acids and propionate and the other are the products of the partial metabolism of glucose in certain tissues and which are transported to the liver and kidney for resynthesis to glucose.

Lactate formed by the oxidation of glucose in skeletal muscle and by erythrocytes, is transported to the liver and the kidney where it reforms glucose. This glucose becomes available again for oxidation in the tissues via the circulation. This process is known as the Cori Cycle or lactic acid cycle (Fig. 10.6).

Glyceride-glycerol of adipose tissue is similarly derived initially from the blood glucose as the free glycerol cannot be utilized readily for the synthesis of triglycerides in this tissue. Glycerides of adipose tissue are subject to hydrolysis continually to form free glycerol, which diffuses out of the tissue into the blood. Gluconeogenesis converts it back to glucose in the liver and kidney. The process continues in a cycle in which glucose is transported from the liver and kidney to adipose tissue from where glycerol is turned back for resynthesis into glucose by the liver and kidney.

Propionate

$$\begin{array}{c} CH_3 \\ | \\ CH_2 \\ | \\ COOH \end{array}$$

CoA-SH

ATP   AMP + PPi

Mg^{++}

$$\begin{array}{c} CH_3 \\ | \\ CH_2 \\ | \\ Co-S-CoA \end{array}$$

Propionyl CoA

CO_2

Propionyl CoA carboxylase

Biotin

ATP   ADP + Pi

$$\begin{array}{c} CH_3 \\ | \\ H-C-COOH \\ | \\ Co-S-CoA \end{array}$$

D-methylmalonyl CoA

Methyl malonyl CoA Racemase

$$\begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ Co-S-CoA \end{array}$$

Methylmalonyl CoA Isomerase

$$\begin{array}{c} CH_3 \\ | \\ HOOC-C-H \\ | \\ Co-S-CoA \end{array}$$
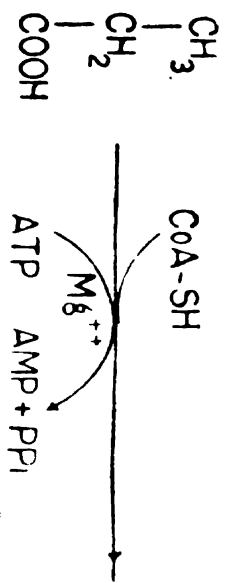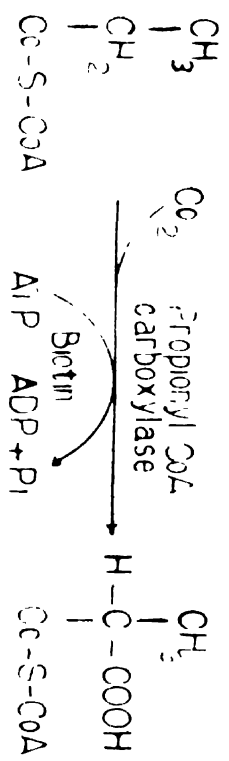
L-methylmalonyl CoA
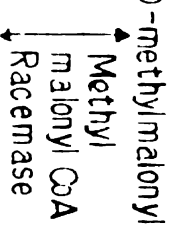
Succinyl-CoA

Intermediates of citric acid cycle

Fig. 10.5

Blood
Glucose

Muscle

Glucose-6-phosphate   Glycogen   Glycogen   Glucose-6-phosphate

Pyruvate ◄────────►Lactate   Lactate   Pyruvate

Lactate

Blood

Pyruvate ◄ ─ ─ ─ ─ ─ ─ ─ ─ ┘
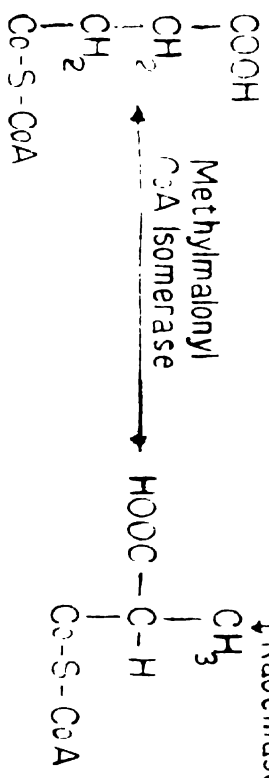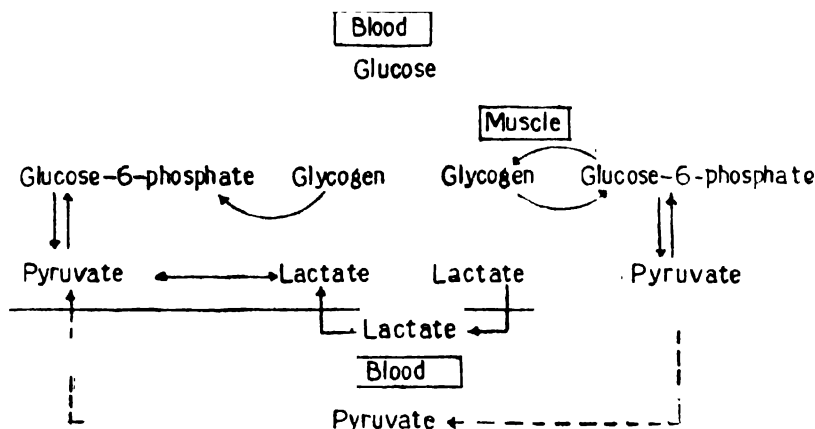
Fig. 10.6

The blood glucose concentration in man varies between 80 and 120 mg per 100 ml and during fasting it may fall to 60 to 70 mg per 100 ml. The level is controlled within the limits under normal conditions. The normal blood glucose level in ruminants is considerably lower, in sheep it is about 40 mg per 100 ml whereas in cattle it is 60 mg per 100 ml. The lower blood glucose level in ruminants is due to the fact that almost the entire dietary carbohydrates are fermented in them to lower fatty acids, which largely replace glucose as the main metabolic fuel of the tissues.

The regulation of the stable levels of glucose in the blood is one of the most finely regulated homeostatic mechanisms in which liver, the extrahepatic tissues, and several hormones play a part. The concentration of blood glucose is an important means to determine the rate of uptake of glucose in both liver and extrahepatic tissues. The hexokinase activity is inhibited by glucose-6-phosphate which indicates that some feed back control may be exerted on glucose uptake in extrahepatic tissues dependent on hexokinase for glucose phosphorylation. This constraint does not exist in the liver since glucokinase is not affected by glucose-6-phosphate.

The hormone insulin plays a central role in the regulation of blood glucose level. The beta cells of the islets of Langerhans in the pancreas secrete insulin into the blood as a direct response to hyperglycemia. Its concentration in the blood parallels that of blood glucose, and prompt hypoglycémia results on administration of insulin.

The anterior pituitary gland secretes hormones to elevate blood sugar thus antagonizing the action of insulin. The growth hormone, ACTH a nd possibly other diabetogenic principles belong to this category.

The adrenal cortex secretes a number of steroid hormones. Gluco-corticoids are important in carbohydrate metobolism as they lead to gluconeogenesis. This is due to increased protein catabolism in the tissues resulting in increased hepatic uptake of amino acids and increased

activity of transaminases and other enzymes concerned with gluconeo-genesis in the liver. Tne gluco-corticoids also inhibit the utilization of glucose in extrahepatic tissues. The gluco-corticoids thus act as insulin antegonist.

The adrenal medulla secretes epinephrine which stimulates glycogen breakdown both in liver and muscle. The stimulation of glycogenolysis by epinephrine is due to its ability to activate the enzyme phosphorylase.

The alpha cells of the islets of Langerhaus of the pancreas produces glucagon which is stimulated by hypoglycemia and causes glycogenolysis by activating phosphorylase in the liver. Unlike epinephrine, glucagon does not have any action on muscle phosphorylase. Gluconeogenesis from amino acids and lactate is also enhanced by glucagon.

The blood sugar is affected by the thyroid hormone. Thyroxine has a diabetogenic action. The kidney exerts a regulatory effect when the blood sugar rises to relatively high levels. The glomeruli filter glucose continually to be returned completely to the blood by the reabsorptive mechanism of the renal tubules. The reabsorption of glucose is effected by phosphorylation in the tubular cells. The reabsorption by the tubule is limited by the concentration of the enzymes responsible for the phosphorylation reaction. When blood glucose levels are elevated, the glomerular filtrate may contain more glucose than can be absorbed; the excess passes into the urine to produce glycosuria. Glycosuria occurs in normal individuals when the venous blood exceeds 170 to 180 mg per 100 ml. This level of venous blood sugar is called the renal threshold for glucose.

Phlorhizin can produce glycosuria experimentally by inhibiting the glucose reabsorption system in the tubule. This is known as renal glycosuria. It may result from inherited defects in the kidney or from disease.

METABOLISM OF FRUCTOSE

The hexokinase enzyme brings about the phosphorylation of fructose to form fructose-6-phosphate in the same manner as it does with glucose or mannose although the affinity of the enzyme for fructose is very small. Fructose-1-phosphate is formed by another enzyme, fructoninase of the liver which catalyses the transfer of phosphate from ATP to fructose. This enzyme is found in kidney and intestine. Glucose is not phosphory-lated by this enzyme, which is not affected by fasting or by insulin. This accounts for the disappearance of fructose from the blood of diabetic patients at a normal rate.

Fructose-1-phosphate aldolase splits fructose-1-phosphate into D-glyceraldehyde and dihydroxyacetone phospate. The hereditary fructose intolerance is caused by the absence of the fructose-1-phosphate aldolase. D-glyceraldehyde is involved in the glycolysis sequence through three possible pathways. One is alcohol dehydrogenase to form glycerol, which

is converted into α-glycerophosphate by glycerokinase. Aldehyde dehydrogenase constitutes the second alternate route where D-glyceraldehyde is transformed into D-glycerate. Liver contains another enzyme, triokinase, which catalyzes the phosphorylation of D-glyceraldehyde to form glyceraldehyde-3-phosphate. This is probably the major pathway for the further metabolism of D-glyceraldehyde. Embden-Meyerhof pathway or the aldolase influence brings about the conversion of the two triose phosphates, dihydroxy acetone phosphate and glyceraldehyde-3-phosphate, to glucose.

The enzyme-1-phosphofructokinase, found in muscle and liver, may also possibly be involved in the phosphorylation directly in position 6 to form fructose-1,6-diphosphate, and intermediate of glycolysis. This does not appear to be the major pathway for fructose metabolism as in that case the hereditary fructose intolerance would not occur.

Injected fructose is not converted to glucose in experimental animals on removal of liver and intestine and hypoglycemia results unless glucose is administered. Fructose only on conversion to glucose in the liver, appears to be available for utilization in significant amounts by brain and muscle. Fructose is more rapidly metabolized by the liver than glucose.

Fructose is metabolized actively by adipose tissue independently of glucose. The adipose tissue appears to utilize fructose more slowly than glucose at low concentrations and at higher concentrations fructose is metabolized at a faster rate than glucose.

Seminal plasma contains free fructose. Glucose may be the precursor of this fructose, the conversion appears to be effected through the sorbitol pathway. Aldose reductase and NADPH catalyse the reduction of glucose to sorbitol which in turn is oxidized to fructose in presence of ketose reductase (sorbitol dehydrogenase) and NAD.

The metabolic pathway of fructose can be indicated as in Fig. 10.7.

METABOLISM OF GALACTOSE

The disaccharide lactose or milk sugar on hydrolysis in the intestine yields galactose, which is readily converted to glucose in the liver. Galactose tolerance test constitutes a test of hepatic function which is based on the ability of the liver to convert galactose to glucose.

Galactokinase with ATP as phosphate donor brings about the phosphorylation of galactose to form galactose-1-phosphate. Uridine diphosphate glucose (UDPG) and the product galactose-1-phosphate react to form uridine diphosphate galactose and glucose-1-phosphate catalyzed by the enzyme, galactose-1-phosphate uridyl transferase. Uridine diphosphate glucose (UDPG) is formed by the enzyme, epimerase. Glucose is finally produced from UDPG as glucose-1-phosphate probably after incorporation into glycogen followed by phosphorylation. This reaction is reversible, glucose can be converted back to galactose which indicates that performed galactose is not essential in the diet. Galactose is required in the body for the formation of milk. It is also a constituent

of glycolipids—cerebrosides, chondromucoids, and mucoproteins.

Glucose is converted to UDP-galactose by the enzyme, galactokinase, in the synthesis of lactose in the mammary gland. Lactose synthetase catalyzes the condensation of UDP-galactose with glucose to form lactose.

Galactosemia is an inherited metabolic disorder which occurs due to the inability to metabolize dietary galactose resulting in the accumulation of galactose in the blood and escaping into urine. Galactose free diets are used to ensure normal growth and development of children affected with the disease.

The pathway by which galactose is converted to glucose is shown in Fig. 10.8.
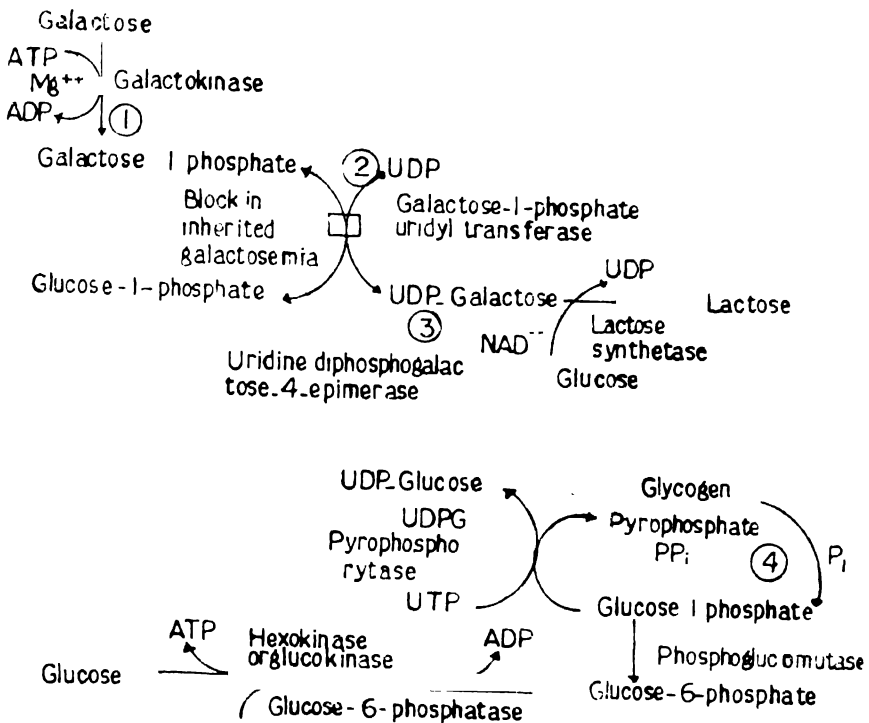
Fig, 10.8

Energy for cellular purposes is derived from the oxidation of carbohydrate, proceeding through a series of intermediate reactions during which much of this energy is transformed into the terminal phosphate bond of ATP molecules. Molecular oxygen is not required in the preliminary breakdown of carbohydrate to pyruvic acid (glycolysis or fermentation), yielding only a small portion of the total potential energy of the carbohydrate molecule. The aerobic oxidation called the tricarboxylic acid cycle, provides the major source of
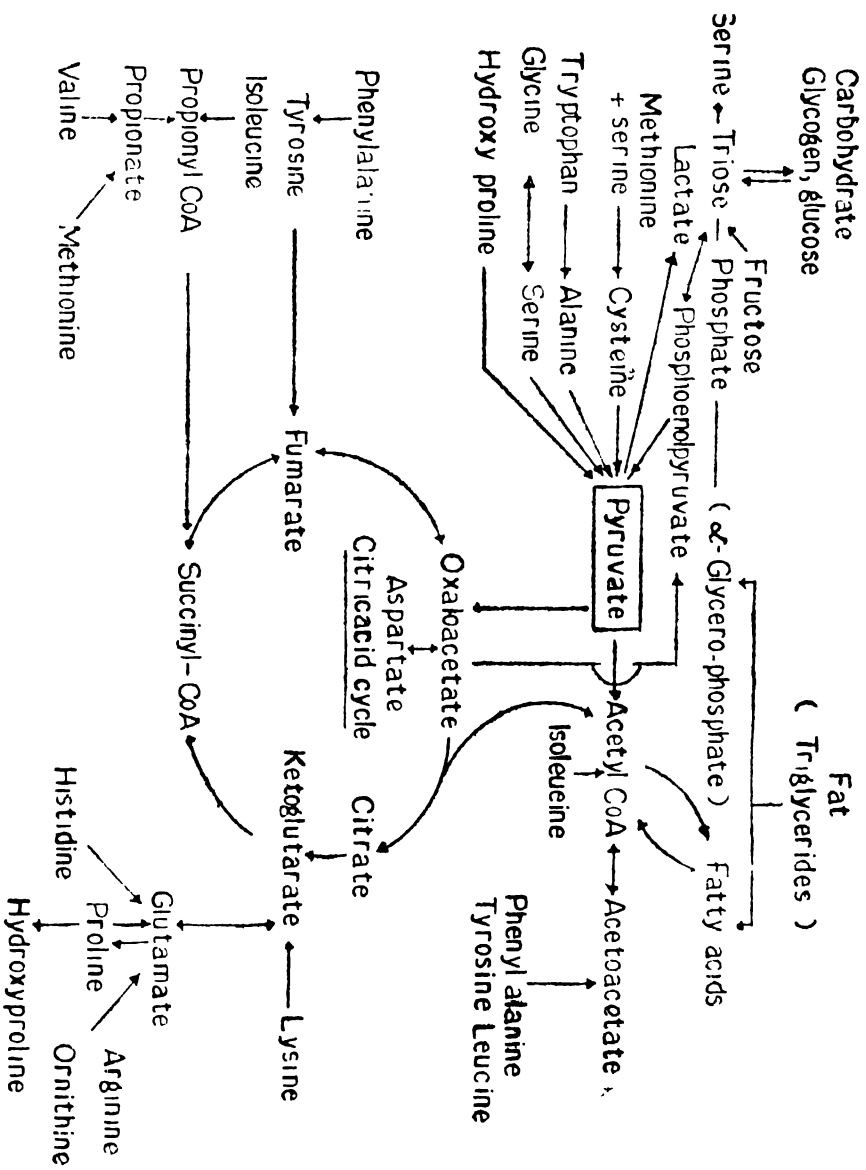
Fig. 10.8

energy. The aerobic oxidation occurs in a stepwise manner with the intermediate participation of hydrogen carriers, the coenzymes; this goes on until the final reaction with molecular oxygen takes place to form water. This electron chain also provides a means for the conversion of the chemical bond energy of carbohydrate fragments into ATP. The enzymes constituting the mitochondrial particles of the cell cytoplasm probably mediate these important reactions. The reactions of the tricarboxylic acid cycle are of additional significance because of the fact that the intermediary metabolic reactions of the lipids and proteins give rise to products which are identical with those of the tricarboxylic acid cycle. This accounts for the interconversion of the three major food stuffs. It explains as to how a high protein diet, low in carbohydrate, can yield sufficient energy for metabolic purposes.

The interconversion of the major foodstuffs is indicated in Fig. 10.8.

Further Reading

S. Dagley and D.E. Nicholson, *An Introduction to Metabolic Pathways* (London: Blackwell, 1970).

David M. Greenberg, *Metabolic Pathways*, Vol. I (London: Academic Press, 1976).

R.A. Field, *The Metabolic Basis of Inherited Disease* (New York: McGraw-Hill, 1960).

H.A. Krebs, 'Gluconeogenesis', *Proceedings of the Royal Society*, 1964, pp. 545–64.

D.A. Pyke, *Disorders of Carbohydrate Metabolism* (London: 1962).

R.H.S. Thompson and I.D.P. Wootton, *Biochemical Disorders in Human Disease* (London: Livingstone, 1970).

H.A. Harper, *Review of Physiological Chemistry* (London: Lange, 1969).

B. Harrow and A. Mazur, *Text Book of Biochemistry* (New York: Saunders, 1958).

E.T. Mertz, *Elementary Biochemistry* (Bombay: Vakils, Feffer and Simons, 1960).

G.H. Bell, J.N. Davidson, and D.E. Smith, *Text Book of Physiology and Biochemistry* (London: Livingstone, 1972).

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